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Inhibitory Potential of A-Amylase and A Glucosidase Enzymes by Bioactive fraction of Bacopa Monnieri: In Vitro study for Anti Diabetic Activity

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ABSTRACT
Diabetes mellitus is a global health concern characterized by hyperglycemia, necessitating innovative therapeutic strategies. Inhibition of carbohydrate-digesting enzymes, particularly a-amylase and a-glucosidase, is a promising approach to control postprandial blood glucose levels. Bacopa monnieri (BM), a renowned medicinal herb with a rich phytochemical profile, has gained attention for its potential anti-diabetic properties. This study explores the inhibitory potential of a bioactive fraction of Bacopa monnieri on a-amylase and a-glucosidase enzymes, offering insights into its role as an in-vitro therapeutic agent for anti-diabetic activity. the preliminary phytochemical screening of leaf extracts of Bacopa monnieri results showed the presence of alkaloids, terpenoids, phenols, carbohydrates and flavonoids. The TLC from solvent systems 7:3 hexane Ethyl acetate, 9:1 chloroform methanol and 9.5:0.5 ethyl acetate methanol showed clear band separation of compounds in all crude fractions. The other characterizations FTIR and LCMS were also carried out. This research sheds light on the promising role of Bacopa monnieri as an in-vitro therapeutic agent for anti-diabetic activity through the inhibition of a-amylase and a-glucosidase enzymes. Further exploration, including in-vivo studies and clinical trials, is warranted to validate its efficacy and safety as a complementary approach in managing diabetes mellitus.

INTRODUCTION

The herbal medicinal plant has been used to treatment or prevention of various human diseases in our countries including and their potential therapeutic effects Halder *et al*.[11] Those plants have used for not only medicinal properties but also their as food beverage, excellent natural human health benefits, pigments and cosmetic things in long years in the worldwide Paul *et al*.[12] Medicinal plants have been distributed to several phytochemical constituents and it's considered to be bioactive resources. Diabetes mellitus (DM) is the carbohydrate metabolic disorders that are characterized by hyperglycemia due to increasing a glucose concentration in the blood Hasanpour *et al*.[11] Diabetes is affected with 578 million of people by 2030 and this figure may expect the increasing in the worldwide around 700 million by 2045 Augustine Daniel *et al*.[4] DM is the classified into type-1 DM or insulin-dependent DM (IDDM), which is affected in the childhood due to little or loss of insulin secretion in the ß-cells of the pancreas Mohamed Othman *et al*.[14] Type-1 DM patient's condition like insulin does not produce in the ß-cells of the pancreas of, because it is attacked by T-cell-mediated autoimmune system. The treatment of the type-1 DM aims to control normal blood glucose levels through insulin therapy, healthy diets as well as every day physical exercise for 30 min Sunday Faith Oyelere *et al*.[17] Another type is the Type-2 DM or non-insulin-dependent DM (NIDDM). This type is mainly affected around 90% of adult. In type-2 DM condition due to body does not produce enough insulin in the ß-cells of the pancreas, which can cause to insulin resistance or insulin deficiency Saravanan Shanmugam *et al*.[22] Uncontrolled high blood sugar levels can lead to various diabetic complications including cardiovascular disease, oxidative stress, diabetic retinopathy blindness, diabetic neuropathy, diabetic nephropathy, renal failure, foot ulcers and need for limb amputation You *et al*.[28] Toluwani Tella *et al*.[24] And Vítor Spínola *et al*.[23]

Bacopa monnieri monnieri as known as Brahmi that is belongs to the family of Plantaginaceae and are tropical plant, which mainly distributed or growing in various countries including Europe, Africa, Asia and southern and Eastern India (especially Tamil Nadu) Rupali Gupta *et al*.[7] The several secondary metabolites are crucially found in the of B. monnieri and it's one of the Indian medicinal plants, which can be briefly used in different biological potential including that anti-epileptic, anti-hypercholesterolemic, bioremediation, anti-microbial, Anti-clastogenic, productive of neurodegenerative diseases, anti-amnesic, plant regeneration and anti-oxidative stress activities Komali *et al*.[13] Kamesh *et al*.[12] Tripathi *et al*.[25] Upadhyaya *et al*.[26] Dipanwita Deb *et al*.[5] Prasansuklab *et al*.[20] Habbu *et al*.[9] Ali *et al*.[2] and Gupta *et al*.[7]

B. monnieri were selected for the current research study based on their several chemical constituents specially those which are known to be rich in the phytochemical constituents like as phenolic and triterpenoids. Hence, in this present study we have to carry out a hexane, ethyl acetate and methanol fractions of B. monnieri that were used to investigate the inhibitory activity of a-amylase and a-glucosidase enzyme activities, which has considered for therapeutic agent against diabetes mellitus.

MATERIALS AND METHODS

Collection of plant materials: The whole plant of the Bacopa monnieri was collected from Mecheri, Salem DT, Tamil Nadu, INDIA in the November and December (38±1°C) in the year 2021. After collected plant leafs were washed with distilled water and shade dried at room temperature for 15 days. After complete drying, the samples were powdered with a grinder. The dried leaf powder (20 g) was sequentially extracted with organic solvents such as hexane, ethyl acetate and methanol. These extract residues were made for concentration to dryness in vacuum rotary evaporator and stored in cold until used.

Extraction of bacopa monnieri: Crude Bacopa monnieri Sample extract was prepared by the Soxhlet extraction method. About 20 gm of powdered sample, material was uniformly packed into a thimble and extracted with 250 mL of different solvents hexane, ethyl acetate and methanolic extract separately. The process of extraction has to be continued for 24 hrs or till the solvent in the siphon tube of extractor becomes colorless. After that, the extract was taken in a beaker kept on a hot plate and heated at 30-40ºC till all the solvent evaporated. The dried extract was kept in the refrigerator at 4ºC till future use Vijayan *et al*.[27]

Screening of preliminary phytochemicals analysis from bacopa monnieri: Preliminary phytochemical analysis was carried out for all the hexane, ethyl acetate and methanolic extract of Bacopa monnieri as per standard methods described by Brain and Turner, 1975 and Evans, 1996.

Characterization of bacopa monnieri

Thin layer chromatography (TLC): The experiment TLC was run on silica gel GF254 pre-coated aluminium sheets, Merck (Germany), for checking the number of compounds as clear spot present in crude plant material. The compounds were detected by irradiating with UV light (254 nm) or reacting with KMnO4 or reacting with 0.25% of ninhydrin or reacting with

iodine vapour. Thin layer chromatography (TLC) is a chromatographic method which is used to separate compounds. It is performed on silica gel GF254 aluminium sheets. Thin film of adsorbent is identified as the stationary silica phase. The combined extracts of Bacopa monnieri hexane, ethyl acetate and methanol were subjected to Thin Layer Chromatography using various solvent systems and observed for characteristic spots under UV light and KMnO4.

FT-IR analysis: FT-IR spectra were recorded with a Perkin Elmer-Spectrum. The spectrometer was continuously purged with nitrogen to eliminate atmospheric water vapour and carbon dioxide. Background spectra, which were collected under the same conditions, were subtracted from the sample spectra automatically. The frequencies for all sharp bands were accurate to 0.001 cm-1. Each sample was scanned under the same condition for four times.

LC-MS analysis: Mass spectrum of the all-crude extracts was recorded on instrument Waters-SynaptG2 by electro spray ionization (ESI) technique with a flow rate of 0.3 mL min-1 on C-18 column and total run time of 45 min. The sample used for recording the mass spectrum was prepared by dissolving 0.2 mg of compound in 10 mL of methanol/acetonitrile.

Assay for inhibitory activity of a-amylase (EC 3.2.1.1): The a-amylase inhibitory activity was determined according to the method of Lourdes Fuentes *et al*.[12] with slight modifications. Various concentration of PSWE was mixed with 100 µl of a-amylase contain 0.5 mg mL^{-1} units in 20 mM sodium phosphate buffer (pH 6.9). The assay mixtures were incubated at room temperature for 30 min. After incubation, added 100 µl of 1% starch solution prepared in 20 mM sodium phosphate buffer (pH 6.9) and assay reactions were incubated at room temperature for 30 min. The inhibition reactions were terminated by adding 1000 µl of 1% 3,5 dinitrosalicylic acid, this incubated in boiling water bath at 60°C for 15 min. Finally, the absorbance was measured at 540 nm using a spectrophotometer (Shimadzu UV-1800, Tokyo, Japan). The inhibition percentage was calculated as following equation:

$$\alpha - \text{amylase inhibition}\,\% = \frac{\text{Abs}_{540}\,\text{control} - \text{Abs}_{540}\,\text{Test}}{\text{Ab}_{540}\,\text{control}}\,\text{x}100$$

Where, Abs_{540} Control is an absorbance of without the a-amylase activity, and Abs_{540} Test is an absorbance of the tested compound. The standard drug acarbose was used as a positive control.

Assay for inhibitory activity of a-glucosidase (EC3.2.1.20): The a-glucosidase inhibitory activity was determined according to the method of Andrea Aleixandre *et al*.[1] with slight modifications. Various concentration of PSWE is mixed with 100 µl of a-glucosidase enzyme 1 units mg^{-1} in 100 mM sodium phosphate buffer at (pH 6.8) and reaction was incubated at 37 °C for 30 min. After incubation, added with solution of 100µl of 10 mM p-Nitrophenyl a, D-phenyl glucopyranoside as a substrate contain with 100 mM sodium phosphate buffer at (pH 6.8). The enzymatic reactions were incubated at 37 °C for 30 min. Finally, the assay reactions were stopped by adding 700 µl of 1 mM sodium carbonate solution. The yellow colour product of p-nitro phenol was released from total enzyme assays and absorbance measured at 410 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan). The inhibition percentage of a-glucosidase was expressed as following equations:

$$\alpha - \text{Glucosidase inhibition}\,\% = \frac{\text{Abs}_{410}\,\text{control} - \text{Abs}_{410}\,\text{Test}}{\text{Ab}_{410}\,\text{control}}\,\text{x}100$$

Where, Abs_{410} Control is an absorbance of a-glucosidase activity without SWE and Abs_{410} Test is an absorbance of a-glucosidase with SWE. The standard drug acarbose was used as a positive control.

Statistical analysis: All the data's obtained for each experimental results were analysis three independent replicates (n = 3). The results were represented as Mean±SEM. All the statistical analysis was employed by Origin 8 pro software.

RESULTS AND DISCUSSIONS

Phytochemical screening: The results of phytochemical screening of Bacopa monnieri leaf extract listed in Table 1. Phytochemical analysis was carried out on the crude leaf extract of Bacopa monnieri which revealed the presence of important bioactive compounds. The presence of phytochemical compounds in the plant Bacopa monnieri was evaluated in leaf using different solvents such as hexane, methanol and ethyl acetate. In the current work the preliminary phytochemical screening of leaf extracts of Bacopa monnieri results showed the presence of alkaloids, terpenoids, phenols, carbohydrates and flavonoids.

Characterization of bacopa monnieri:

Tlc studies on various leaf extract of bacopa monnieri: The TLC studies obtained for various extracts. TLC studies on all crude extract of Bacopa monnieri showed various separations of spots, in different solvent system. The TLC from solvent systems 7:3 hexane Ethyl acetate, 9:1 chloroform: methanol and 9.5:0.5 ethyl acetate methanol showed clear band separation of compounds in all crude fractions.

FT-IR spectral studies for hexane, ethyl acetate and methanol fraction: The region of FT-IR radiation helps

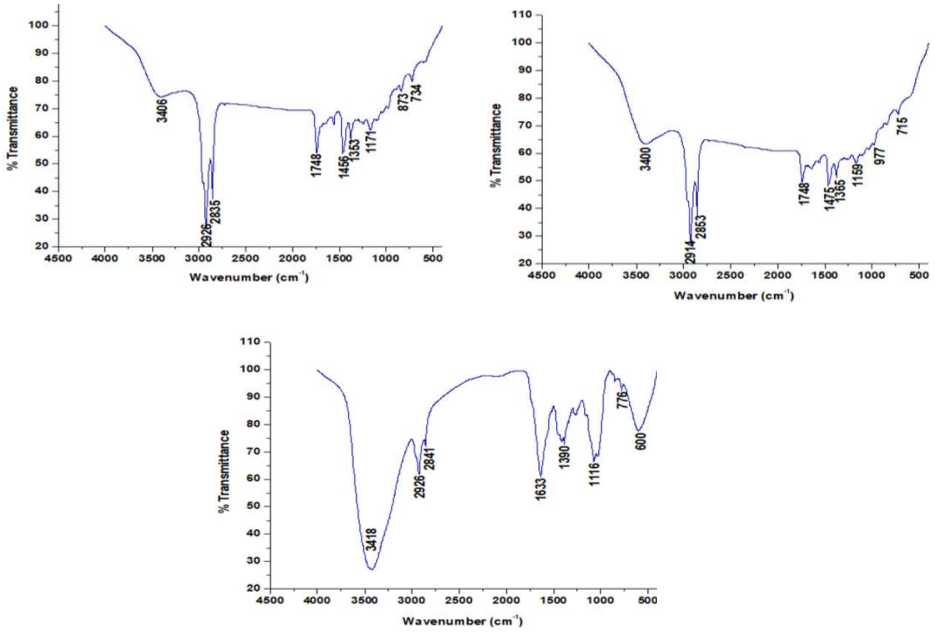

Fig. 1: FT-IR spectral studies for hexane, ethyl acetate and methanol fraction

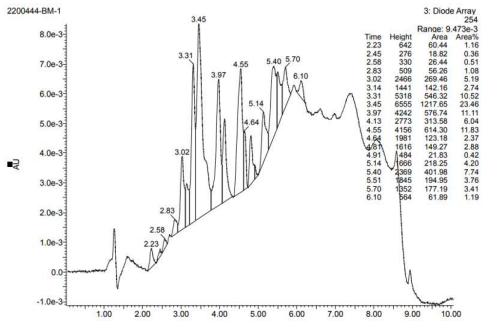

Fig. 2: LC-MS analysis of Hexane Extract

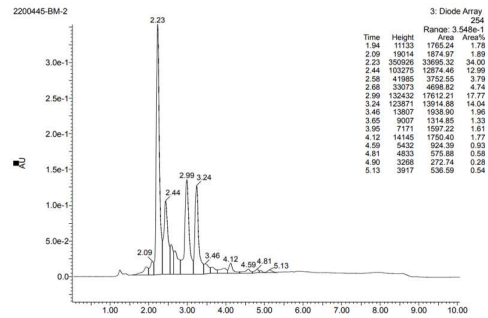

Fig. 3: LC-MS analysis of Ethyl acetate Extract

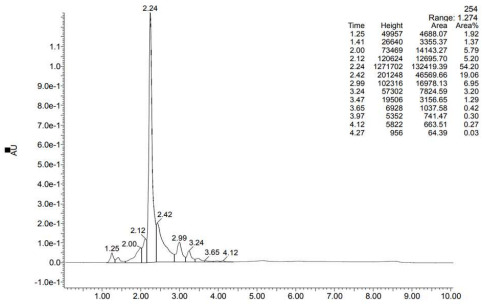

Fig. 4: LC-MS analysis of Methanol Extract

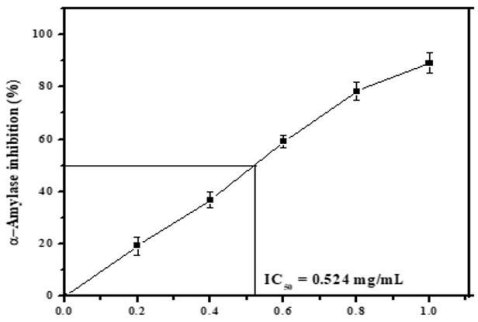

Fig. 5: Inhibitory activity of ethyl acetate fraction of B. monnieri on a-amylase enzyme activity

to find the functional groups of the active components present in all crude extracts based on the peak's values of the FT-IR spectrum. When the crude extracts were passed into the FT-IR the functional groups of the components were separated based on its peak's ratio. The results of FT-IR analysis confirmed the presence of carbonyl, aromatic, alkane, phenol and alcohol-based compounds. The peak at 3406, 3400 and 3418 cm-1 assigned to the O-H stretching vibration. In addition, the peak at 1748-1633 cm-1 assigned to the C = O stretching vibration of carbonyl compounds present in

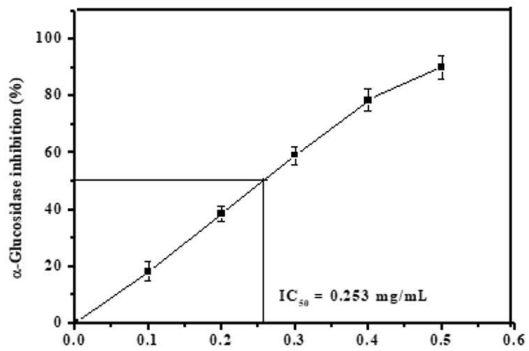

Fig. 6: Inhibitory activity of ethyl acetate fraction of B. monnieri on a-glucosidase enzyme activity

the various extracts. The OH bending vibrations are seen between the ranges 1390-1353 cm-1. The FT-IR spectra of the hexane, ethyl acetate and methanol fraction from B. monnieri showed Fig. 1, respectively.

LC-MS Studies of hexane, ethyl acetate and methanol extract: LC-MS analysis of hexane, ethyl acetate and methanol extracts of Bacopa monnieri leaf had detected various components of peaks, these results were showed in Figure 2, 3 and 4 respectively. The case of hexane extract, the different compounds (Major) peaks were found with retention time 3.45, 3.97 and 4.55 showed the mass (m/z) 287, 613 and 277, respectively. The mass spectrum of ethyl acetate extract showed major compounds at retention time at 2.23, 2.99 and 4.55 corresponding to the mass (m/z) 325, 197 and 271, respectively. In the methanol extract the compounds with mass (m/z) 325 and 339 identified as major component with retention time 2.24 and 2.42, respectively.

A-amylase enzyme inhibition by ethyl acetate fraction of Bacopa monnieri: In order to identify the potent natural inhibitor against a-amylase enzyme, the ethyl acetate fraction of B. monnieri was evaluated the inhibition of a-amylase enzyme activity and result was showed in Fig. 5. From the graph, it is clearly indicated that the ethyl acetate fraction of B. monnieri inhibited a-amylase enzyme activity in dose-dependent manner (0.2-1.0 mg mL^{-1}). The ethyl acetate fraction of B. monnieri at the concentration of 1.0 mg mL^{-1} significantly inhibited the a-amylase enzyme activity at 88.33%. The IC50 value of ethyl acetate fraction of B. monnieri against a-amylase activity was found to be 0.524 µg mL^{-1}, when compared with acarbose 130 µg mL^{-1}, respectively.

A-glucosidase enzyme inhibition by ethyl acetate fraction of Bacopa monnieri: The ethyl acetate fraction of B. monnieri was studied for potential inhibition against a-glucosidase enzyme activity and shown in Figure 6. The graph clearly indicated that the ethyl acetate fraction of B. monnieri inhibited against a-glucosidase enzyme activity in increase concentration (i.e. dose-dependent manner) from 0.1-0.5 mg mL^{-1}, respectively. The ethyl acetate fraction of B. monnieri at 0.5 mg mL^{-1} concentration showed significantly highest a-glucosidase enzymes inhibition percentage was found to be 90.12%. The inhibition constant IC50 value for ethyl acetate fraction of B. monnieri was found to be 0.253 µg mL^{-1} and of standard drug acarbose to be 125 µg mL^{-1}.

In vitro determination of a-amylase and a-glucosidase enzyme inhibition revealed that the ethyl acetate fraction of B. monnieri. The inhibition effects of acarbose commercially available drugs have been used for inhibition study of a-amylase and a-glucosidase enzyme activity. Acarbose plays an important role in the control or prevention of postprandial hyperglycemia Lakshmana Senthil *et al*.[14] Acarbose, miglitol and voglibose, these are the currently available drugs for inhibition of a-glucosidase enzyme and antidiabetic therapeutic agent and its commonly side effects like abdominal discomfit, flatulence and diarrhoea Zhiyang Liu *et al*.[15] The search for natural bioactive compounds from medicinal plants which is nontoxic is ever increasing. The a-amylase and a-glucosidase inhibition were screened from ethyl acetate fraction of B. monnieri, that results were obtained anti-diabetic potential when compared hexane and methanol fractions. The acetone extracts of Undaria pinnatifida showed the highest percentage of inhibition (69.3±0.5%) amongst the tested seaweed extracts, followed by methanolic extracts of Laminaria digitata with an inhibition of 61.5±0.7% at the concentration of 2 mg mL^{-1} Zaharudin *et al*.[30]

Previous study has been reported that the highest inhibition performance was for extracts from Lessonia trabeculate, with a value of 69.75±3.49% observed for the methanol in fraction and 34.69±2.31% for the conventional fraction and this was much higher than those obtained for the other three species Yuan *et al*.[29] Nazikussabah *et al*.[31] has been reported more inhibition effective of acetone extract of Undaria pinnatifida and Laminaria digitata on a-glucosidase activity. Previous study has been reported that the inhibitory activity of phlorotannins isolated compounds (1-5) from Ecklonia cava on a-glucosidase. Compounds 3 and 5 displayed the most potent inhibitory activity, with IC50 values of 2.3±0.1 and 2.3±1.2 µM, respectively. Compound 1 showed moderate inhibitory activity, with IC50 values of 12.5±3.1 µM, whereas compounds 2 and 4 suppressed the catalytic reaction of a-glucosidase at high concentrations, with IC50 values of 32.5±2.1 and 59.8±0.8 µM, respectively Sae Rom Park *et al*.[18] The ethyl acetate fraction of B.

Table 1: Screening test for phytochemicals analysis in the various extracts of hexane, ethyl acetate and methanol from Bacopa monnieri

Phytochemical Test	Hexane	Ethyl acetate	Methanol
Alkaloid test			
A) Drangen droff	+	+	-
B) Hagers test	+	+	-
C) Wagner's test	+	+	-
Test for terpenoids			
A) H_2SO_4 test	-	+	-
Test for phenols and tannins			
A) Lead acetate test	-	+	
B) K_2CO3	-	++	+
C) Gelatin	-	+	
Protein and amino acid			
A) $CuSO_4$	-	-	
B) Ninhydrin	-	-	
Carbohydrate			
A) Molish test	-	-	+
B) Benedict test	-	++	
Saponin	-	-	-
Flavonoids	-	+	+

monnieri was evaluated for more inhibition effectives on a-glucosidase activity. Hence, Natural bioactive fractions were presented in the medicinal plant of B. monnieri which has considered for anti-diabetic molecules.

CONCLUSION

The inhibitory potential of Bacopa monnieri (BM) on a-amylase and a-glucosidase enzymes presents a compelling prospect in the quest for effective anti-diabetic agents. This study, focusing on the bioactive fraction of BM, has provided valuable insights into its role as an in-vitro therapeutic agent for anti-diabetic activity. BM, renowned for its diverse phytochemical composition, including alkaloids, saponins, flavonoids and polyphenols, harbours the potential to modulate biological processes. Of particular significance are the bacosides, unique triterpenoid saponins found in BM, which have demonstrated remarkable bioactivity. The in-vitro experiments conducted in this study have demonstrated that the bioactive fraction of BM exerts a substantial inhibitory effect on both a-amylase and a-glucosidase enzymes. This inhibition is of paramount importance in the context of diabetes management, as it can significantly impede carbohydrate digestion and glucose absorption.

While the in-vitro results are promising, further research is imperative. In-vivo studies, including animal models and clinical trials, are necessary to validate the safety, efficacy and optimal dosage of BM-derived bioactive fractions for anti-diabetic applications. Comprehensive toxicological assessments will contribute to the safety profile of BM-based interventions. In conclusion, the bioactive fraction of Bacopa monnieri demonstrates substantial inhibitory potential against a-amylase and a-glucosidase enzymes, positioning it as a promising in-vitro therapeutic agent for anti-diabetic activity. The multifaceted phytochemical composition of BM, with bacosides as key players, holds great potential for advancing diabetes management strategies. Further research and clinical validation are crucial steps toward harnessing the full therapeutic potential of this natural remedy in the fight against diabetes mellitus.

REFERENCES

1. Aleixandre, A., M. Gisbert, J. Sineiro, R. Moreira and C.M. Rosell, 2022. In vitro inhibition of starch digestive enzymes by ultrasound-assisted extracted polyphenols from Ascophyllum nodosum seaweeds. J. Food Sci., 87: 2405-2416.
2. Ali, D., K.E. Ibrahim, S.A. H ussain and M.M. Abdel-Daim, 2020. Role of ros generation in acute genotoxicity of azoxystrobin fungicide on freshwater snail lymnaea luteola l. Environ. Sci. Pollut. Res., 28: 5566-5574.
3. Brain, K. R. and T. D Turner, 1995. Practical evaluation of phytopharmaceuticals. Bristol. Edn., Scientechnica, Pages: 144.
4. Daniel, A.I., T.Y. Gara, Y.O. Ibrahim, F.M. Muhammad, F.E. Salisu, R. Tsado and A.M. Agboola, 2022. In vivo antidiabetic and antioxidant activities of chloroform fraction of nelsonia canescens leaf in alloxan-induced diabetic rats. Pharmacol. Res. Mod. Chin. Med., Vol. 3. 10.1016/j.prmcm.2022.100106
5. Deb, D.D., P. Kapoor, R.P. Dighe, R. Padmaja and M.S. Anand., 2008. In vitro safety evaluation and anticlastogenic effect of bacomind on human lymphocytes. Biomed. Environ. Sci., 21: 7-23.
6. Evans, W. C., 1996. Trease and Evans Pharmacognosy. 14th. Ed. Edn., Saunders Ltd., Pages: 159.
7. Gupta, R., A. Singh, P.V. Ajayakumar and R. Pandey, 2017. Microbial interference mitigates meloidogyne incognita mediated oxidative stress and augments bacoside content in bacopa monnieri L. Microbiol. Res., 199: 67-78.

8. Gupta, R., A. Singh, M. Srivastava, K. Shanker and R. Pandey, 2019. Plant-microbe interactions endorse growth by uplifting microbial community structure of bacopa monnieri rhizosphere under nematode stress. Microbiol. Res., 218: 87-96.
9. Habbu, P., S. Madagundi, R. Kulkarni, S. Jadav, R. Vanakudri and V. Kulkarni, 2013. Preparation and evaluation of bacopa phospholipid complex for antiamnesic activity in rodents. Drug. Invent. Today., 5: 13-21.
10. Halder, S., U. Anand, S. Nandy, P. Oleksak and S. Qusti *et al*., 2021. Herbal drugs and natural bioactive products as potential therapeutics: A review on pro-cognitives and brain boosters perspectives. Saudi. Pharm. J., 29: 879-907.
11. Hasanpour, M., M. Iranshahy and M. Iranhahi, 2020. The application of metabolomics in investigating anti-diabetic activity of medicinal plants. Biomed. Pharmacother., Vol. 128. 10.1016/j.biopha.2020.110263
12. Kamesh, V. and T. S umathi, 2012. Antihypercholesterolemic effect of bacopa monniera linn. on high cholesterol diet induced hypercholesterolemia in rats. Asian. Pac. J. Trop. Med., 5: 949-955.
13. Komali, E., C. Venataramaiah and W. Rajendra, 2021. Antiepileptic potential of bacopa monnieri in the rat brain during ptz-induced epilepsy with reference to cholinergic system and atpases. J. Trad. Compl. Med., 11: 137-143.
14. Senthil, S.L., T.V. Kumar, D. Geetharamani, G. Suja, R. Yesudas and A. Chacko, 2015. Fucoidan-an a-amylase inhibitor from sargassum wightii with relevance to niddm. Int. J. Bio. Macromol., 81: 644-647.
15. Liu, Z., X. Li, F. Sun, T. Zhou and Y. Zhou, 2017. Overexpression of oscipk30 enhances plant tolerance to rice stripe virus. Front. Microbiol., Vol. 8 .10.3389/fmicb.2017.02322
16. Othman, M.S., A.M. Khaled, A.H. Al-Bagawi, M.A. Fareid, R.A. Ghany, O.A. Habotta and A.E.A. Moneim, 2021. Hepatorenal protective efficacy of flavonoids from ocimum basilicum extract in diabetic albino rats: A focus on hypoglycemic, antioxidant, anti-inflammatory and anti-apoptotic activities. Biomed. Pharmacother., Vol. 144 .10.1016/j.biopha.2021.112287
17. Oyelere, S.F., O.H. Ajayi, T.E. Ayoade, G.B.S. Pereira, B.C.D. Owoyemi, A.O. Ilesanmi and O.A. Akinyemi, 2022. A detailed review on the phytochemical profiles and anti-diabetic mechanisms of momordica charantia. Heliyon., Vol. 8 .10.1016/j.heliyon.2022.e09253
18. Park, S.R., J.H. Kim, H.D. Jang, S.Y. Yang and Y.H. Kim, 2018. Inhibitory activity of minor phlorotannins from ecklonia cava on a-glucosidase. Food. Chem., 257: 128-134.
19. Paul, S., S. Chakraborty, U. Anand, S. Dey and S. Nandy *et al*., 2021. Withania somnifera (L.) dunal (ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects. Biomed. Pharmacother., Vol. 143. 10.1016/j.biopha.2021.112175
20. Prasansuklab, A., J.M. Brimson and T. Tencomnao, 2020. Potential thai medicinal plants for neurodegenerative diseases: A review focusing on the anti-glutamate toxicity effect. J. Trad. Compl. Med., 10: 301-308.
21. Fuentes, L.R., C. Richard and L. Chen, 2021. Sequential alcalase and flavourzyme treatment for preparation of a-amylase, a-glucosidase, and dipeptidyl peptidase (dpp)-iv inhibitory peptides from oat protein. J. Funct. Foods., Vol. 87. 10.1016/j.jff.2021.104829
22. Shanmugam, S., P. Thangaraj, B.D. Lima, G.G.G. Trindade and N. Narain., 2020. Protective effects of flavonoid composition rich p. subpeltata ortega. on indomethacin induced experimental ulcerative colitis in rat models of inflammatory bowel diseases. J. Ethnopharmacology., Vol. 248. 10.1016/j.jep.2019.112350
23. Spínola, V., E.J. Llorent-Martínez and P.C. Castilho, 2020. Inhibition of a-amylase, a-glucosidase and pancreatic lipase by phenolic compounds of rumex maderensis (madeira sorrel). influence of simulated gastrointestinal digestion on hyperglycaemia-related damage linked with aldose reductase activity and protein glycation. LWT., Vol. 118 .10.1016/j.lwt.2019.108727
24. Tella, T., B. Masola and S. Mukaratirwa, 2022. Anti-diabetic potential of psidium guajava leaf in streptozotocin induced diabetic rats. Phytomed. Plus., Vol. 2 .10.1016/j.phyplu.2022.100254
25. Singh, A., P. Tripathi, S. Nigoskar, R. Singh and S. Kala., 2022. Role of peripheral blood parameters (neutrophil-to-lymphocyte, lymphocyte-to-monocyte and neutrophil-to-monocyte ratios) in breast cancer. Curr. Med. Res. Pract., 12: 67-72.
26. Upadhyaya, C., T. Upadhyaya and I. Patel, 2022. Exposure effects of non-ionizing radiation of radio waves on antimicrobial potential of medicinal plants. J. Radiat. Res. Applied Sci., 15: 1-10.
27. Vijayan, R., S. Joseph and B. Mathew, 2017. Indigofera tinctoria leaf extract mediated green synthesis of silver and gold nanoparticles and assessment of their anticancer, antimicrobial, antioxidant and catalytic properties. Artif. Cells. Nanomed. Biotechnol., 46: 861-871.
28. You, Q., F. Chen, X. Wang, Y. Jiang and S. Lin, 2012. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT. Food. Sci. Technol., 46: 164-168.

29. Yuan, Y.G., S. Zhang, J.Y. Hwang and I.K. Kong, 2018. Silver nanoparticles potentiates cytotoxicity and apoptotic potential of camptothecin in human cervical cancer cells. Oxidative. Med. Cell. Longevity., 2018: 1-21.
30. Zaharudin, N., A.A. Salmeán and L.O. Dragsted, 2018. Inhibitory effects of edible seaweeds, polyphenolics and alginates on the activities of porcine pancreatic a-amylase. Food. Chem., 245: 1196-1203.
31. Zaharudin, N., D. Staerk and L.O. Dragsted, 2019. Inhibition of a-glucosidase activity by selected edible seaweeds and fucoxanthin. Food. Chem., 270: 481-486.