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Investigating The Therapeutic Potential of Propolis Extract Against Diabetes-Associated Enzymes

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Abstract:

Recently, herbal products and supplements have beenused tremendously for health care.Propolis, a byproduct secreted by honey beesfor protecting the hive from fungal infections is used in our current study to identify the biochemical compounds by GCMS analysis and to evaluate the antidiabetic activity of Propolis. Aqueous and phenolic extracts of Propolis were selected for the enzyme inhibition activity against alpha-amylase and alpha-glucosidase. GCMS analysis fetches the presence of forty various compounds and among thosea few compounds possess high biological activities like Hexadecanoic acid, Dinaphtho[2,1-b:1',2'-d]furan and Chrysin. These compounds were reported to possess different therapeutic effects from earlier studies. In vitro α-amylase and α-Glucosidase activity was carried out with the help of aqueous and phenolic extract of propolis andthe percentage inhibition of assays showed maximum inhibitory effects were Phenol extract of Propolis against alpha-amylase inhibited 7.62%, 13.2%, 24.5%, 38.5 % and 49.9% respectively. In the same way, the aqueous extract of propolis inhibits alpha-amylase8.7%, 15.7%, 29.3%, 48.7% and 61.7% in various concentrations. respectively. Similarly, the phenolic and aqueous extracts reacted against alpha-glucosidase in various concentrations the inhibitory results are 8.6%, 15.0%, 27.7%, 44.3 % and 51.3% respectively. In the same way, the aqueous extract of propolis shows 10.7%, 18.5%, 37.3%, 56.0% and 67.7% respectively.In vitro α-amylase and α- Glucosidase activity has shownthat the aqueous propolis extract exhibits a higher inhibitory effect when compared to that of phenolic extract.This study suggests the aqueousextract of Propolis might be considered as a potential source of bioactive constituents with excellent antidiabetic activity.

Keywords: Propolis, Anti-diabetic activity, alpha-amylase, alpha-glucosidase.

INTRODUCTION:

One of the most significant global public health issues is diabetes mellitus, which is characterized by an abnormal increase in blood glucose levels. As a result of improper glucose homeostasis, fluctuations in metabolism lead to limitation of insulin secretion

[1].Diabetes is categorised into numerous types, namely Insulin-dependent diabetes mellitus (IDDM), non-insulin-dependent diabetes mellitus (NIDDM), maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes caused by endocrinopathies, steroid usage [2].According to International Diabetes Federation (IDF) research, raised blood glucose is the third highest risk factor for early death worldwide, followedby high blood pressure and smoking [3]. Chronic complications of diabetes lead to cardiovascular diseases, neuropathy, nephropathy, and retinopathy[4].

NIDDM involves a more complex interaction between genetics and lifestyle. There is clear evidence that NIDDM has a stronger inheritance profile than IDDM. Most patients with this disease have at least one parent with NIDDM [5].Rapid digestion of carbohydrates is the primary factor in the emergence of hyperglycemia that is associatedwith chronic disorders, termed postprandial glycemia [6].Controlling post-prandial hyperglycemia by avoiding the absorption of glucose by inhibiting the digesting enzymes that break down food like alpha-amylase and alpha-glucosidase is a significant therapeutic option for treating NIDDM [7]. Inhibitory agents play a vital role in postponing the absorption of glucose by blocking the digestive enzymes and maintainingthe plasma glucose level, such agents include synthetic drugs and medicinal plants. There are many synthetic drugs available commercially with numerous side effects that include diarrhoea and other intestinal disturbances such as bloating, flatulence, cramping, and abdominal pain[8]. In the current study, Propolis a bee product is used as an inhibitory agent to inhibit the digestive enzyme and to control the blood glucose level. In the previous stage of Honey, a resinous substance with which the beesimpressment of interstice of their hives, bee glue is termed as Propolis[9]. Propolis is acclaimed to have antiseptic, antibacterial, antimycotic, astringent, spasmolytic, anti-inflammatory, anaesthetic and antioxidant, antitumoral, anti-fungal, anti-ulcer, anticancer and immunomodulatory effects[10]. In modern days people prefer to consume food thatcan provide nutrition as well as a supplementary diet. In connection with this, the present study was designed that propolis can be consumed as a supplementary diet thatcan provide nutrition and the objective speaks about the antidiabetic activity of propolis.

Materials and Method:

Biochemical analysis of Propolis:

The biochemical analysis was carried out for the aqueous extract of the Propolis powder to identify the presence of tannin, saponin, resin, flavonoids Alkaloids, Steroids, Terpenoids, Glycosides, Protein, carbohydrates, Quinone, Acid[11] [12] [13] [14].

GCMS Analysis:

GC-MS analysis was carried out on anAgilent GC 7890A/MB5MS equipped with a DB-5ms Agilent fused silica capillary column (30 × 0.25mm ID; film thickness: 0.25 μm), operating in electron impact mode at 70 eV. Pure helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 1 μL was employed (the split ratio is 10: 1). Mass transfer line and injector temperature were set at 230 and 250^0C, respectively. The oven temperature was programmed from 70 (isothermal for 3min) to 300^oC(isothermal for 9min) at the rate of 10^oC/min. The totalGC running time was 34min and the MS detection was completed within 35min.

By GC-MS, the compounds were separated and then they were eluted from the column and made enter into the detector which was capable of creating an electronic signal. Then they were processed by the computer to generatea chromatogram. Then the compound entered into the electron ionization and detector, where they were bombarded with a stream of electrons causing them to break apart into fragments, they were charged ions and were calibrated from the graph, called the mass spectrum, and is the fingerprint of the molecule.

Inhibition of α- Amylase Enzyme:

α-amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500 µg/ml) to which 1% of starch solution and 100 µl of 0.2 mm of phosphate buffer (pH -6.9) were added. The reaction was allowed to be carried out at 37°C for 5 min and terminated by the addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. the α-amylase activity was determined by measuring colourintensity at 540 nm in a spectrophotometer[15].

Inhibition of α-glucosidases Enzyme

The inhibitory activity was determined by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M tris buffer (pH 8) and various concentrationsof sample (100-500 mg/ml). The reaction mixture was incubated at 37°C for 10 min. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was terminated by the addition of 2 ml of 6 N HCl. The intensity of the colour was measured at 540 nm in a spectrophotometer.

The results were expressed as % inhibition using the formula:

$$\% \text{ inhibitory activity} = (Ac-As)/Ac \times 100$$

Where Ac is the absorbance of the control and A

s is the absorbance of the sample.

The inhibitory concentration (IC50) value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The IC50 values were determined

from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values[15].

Statistical Analysis:

All the obtained data were log-transformed for the statistical analysis. Replicates of mean value were tested by Analysis of variance (ANOVA). The entire statistical test was performed by SPSS software version 20.

Results and Discussion

Table 1 indicates the GC-MS study of honey bee propolis which reveals that certain compounds are found to be high like Hexadecenoic acid, Dinaphtho[2,1-b:1',2'-d]furan, and Chrysin which plays a major role in other biological activities.

The biomolecules given in Table1containhigh affinity in the mitigation of chemotherapy side effects, and the ability to modulate cardiovascular diseases, and arthritis due to the presence of anti-inflammatory properties [16]. Propolis contains an antimicrobial property that has a high potential to fight against harmful microorganismsand pathogens which tend to cause various diseases[17][18].

Table 1: List of Compounds that possesses anti-diabetic activity identified in GCMS analysis

S.No	Compoud Name	Peak Area (%)	Retained Time	Molecular Formula	Molecular Weight (g/mol)	Biological activity
1.	Hexadecanoic acid	9.65	18.252	$C_{16}H_{32}O_2$	256.42	Antioxidant activity
2.	Dinaphtho[2,1-b:1',2'-d]furan	11.45	23.829	$C_{20}H_{12}O$	268.3	Antimicrobial activity
3.	Chrysin	66.43	24.578	$C_{15}H_{10}O_4$	254.241	Antioxidant and anti-inflammatory property

of Propolis:

Figure 1 shows Hexadecenoic acid is a fatty acid that can examined in all solvent's studies have reported that hexadecenoic acid possesses antioxidant, Hypercholesteremic, nematicide and pesticidal activity [19]. Studies revealed that Figure 2 Dinaphtho[2,1-b:1',2'-d]furanexhibits strong inhibitory activity on human hepatocellular carcinoma cell lines (HepG2 and SMMC-7721 cells), uterine cervix cancer Hela cells and acute promyelocytic leukemia NB4 cells [20].Propolis is high in flavonoid Chrysin constituent'snumerous biological properties that includeantitumor[21],anti-diabetic property[22],antimicrobial property[23][24] , anti-allergic property [25] and antioxidant properties[26].

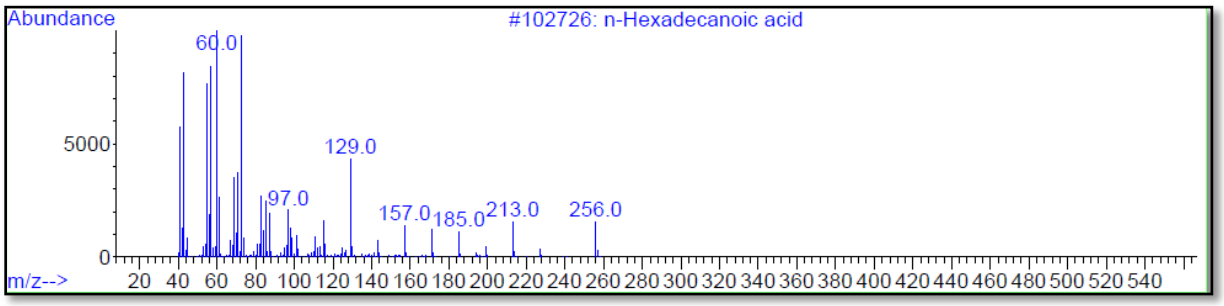

Figure 1 :Hexadecanoic acid

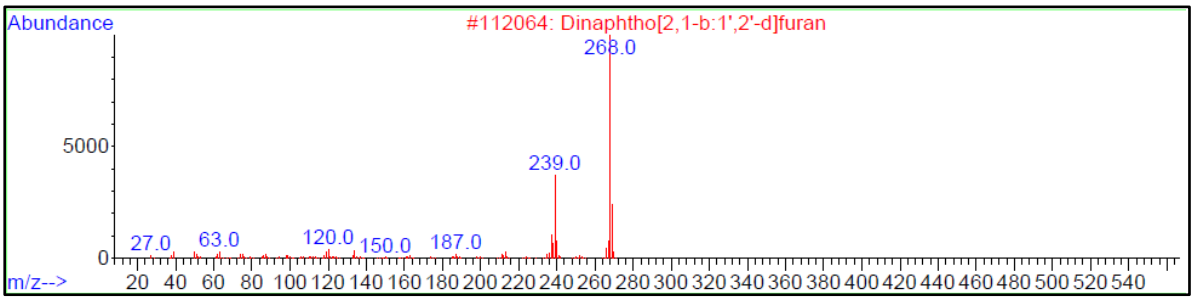

Figure 2 :Dinaphtho[2,1-b:1',2'-d]furan

Table 2 and Figure 4 show the inhibition percentage of phenol and aqueous extract of Propolis and miglitol as standard were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by the *in-vitro* method. The phenolic and aqueous extract of propolis was taken in different concentrations of 50,100,200,400,600 μg/ml were phenolic extract exhibited 7.62%, 13.2%, 24.5%, 38.5 %, 49.9% α-amylase activity and 8.7%, 15.7%, 29.3%, 48.7% and 61.7% α-glucosidase activity respectively. Whereas, Propolis aqueous extract exhibited 9.6%, 10.0%,34.6%,7.6%, and 38.6%, α-amylase activity possesses good anti-diabetic activity. Hence, from the study, the results we interpret those aqueous extracts of propolis showed excellent inhibition proving the anti-diabetic activity when compared with phenolic extract of propolis.

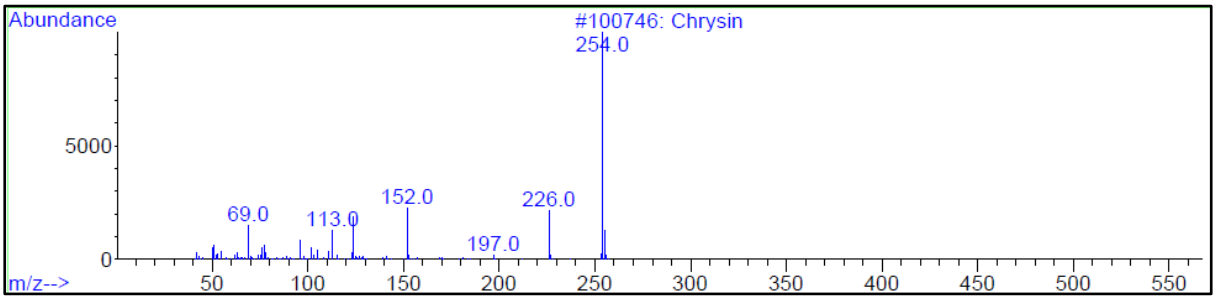

Figure 3: Chrysin

Table 2: Shows the IC_{50}values for in vitro α-amylase and α-glucosidase inhibition by extracts of Propolis.

Sample	Concentration	IC_{50} α-Amylase	IC_{50} α- glucosidase
Miglitol	50 100 200 400 600	197.40 ±1.0	138.3333±1.0
Phenol Extract	50 100 200 400 600	583.1333 ±1.0	535.5385 ±1.0
Aqueous Extract	50 100 200 400 600	450.3229 ± 1.0	388.1553±1.0

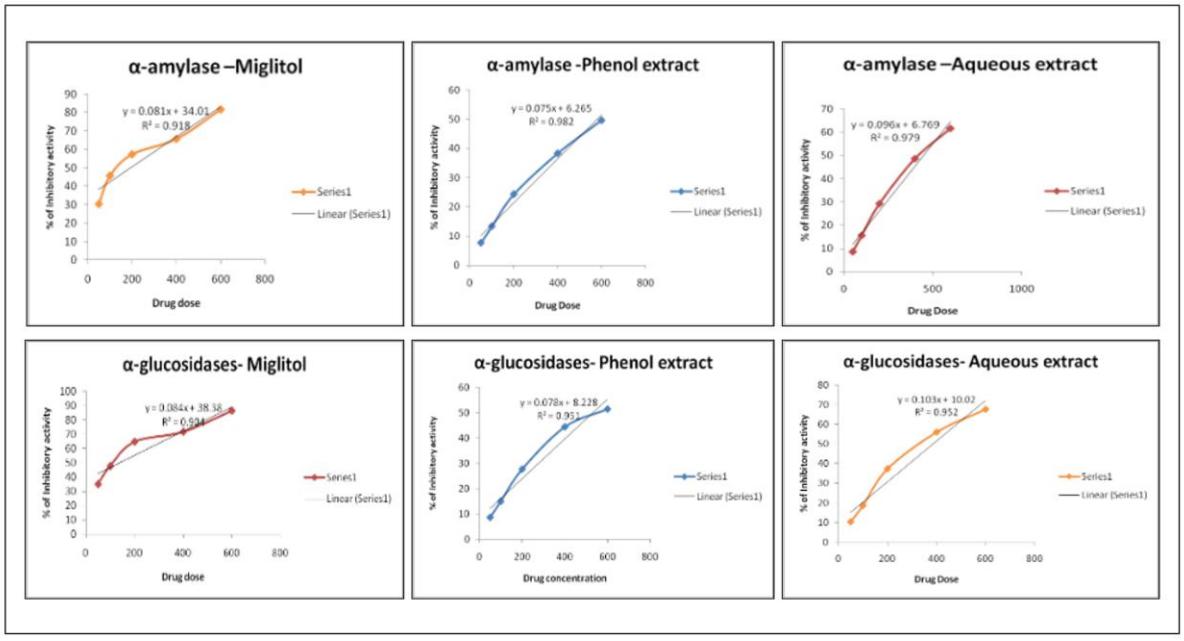

Figure 4: Enzyme inhibition of Phenol and Aqueous extracts of Propolis

Many herbal extracts have been reported to have anti-diabetic properties and are utilised in Ayurvedic medicine to treat diabetes. Many contemporary medications have been

prepared using herbal extracts, either directly or indirectly**.** In this study, an *in vitro* inhibitory effect of different extracts of *Propolis on* alpha-amylase and alpha-glucosidase activities was evaluated.Diabetes always flounders like polyuria, polydipsia, retinopathy, fatigue and macrovascular complications refer to increased atherosclerosis-related events such as myocardial infarction and stroke[27][28][29]. The slant in controlling hyperglycaemia in people suffering from diabetes is to impede or relent absorption of carbohydrates after food intake. The enzyme α-glucosidase plays a role in the breakdown of Complex starches, oligosaccharides, and disaccharides into simple sugars prior the absorption in the duodenum and upper jejunum[30].Inhibition of the α-glycosidase enzyme decelerates the absorption of carbohydrates from the GI tract and helps in the decrease of the rate of rise in postprandial glucose (PP hyperglycemia).

This slack off the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycaemic index control in people with diabetes Miglitol is α-glucosidase inhibitor which reduces digestion of complex carbohydrates and slows their absorption from the gut[31]. These drugs also increase the release of the glucoregulatory hormone glucagon-like peptide-1 into the circulation, which may contribute to their glucose-lowering effects[32]. However, they may cause side effects such as malabsorption, abdominal pain, flatulence, anddiarrhoea which lead to a high discontinuation rate[33]. Acarbose and miglitol should not be prescribed to individuals with renal impairment. Experimental results showed that both extracts significantly inhibited the α-glucosidase and α-amylase enzymes. Aqueous extract showed better α-glucosidase inhibitory activity. Phenol extract showed less inhibitory activity than aqueous extract revealing thatHexadecanoic acid possesses antioxidant activity [34]and similarly, Dinaphtho[2,1-b:1',2'-d]furan has antimicrobial activity[35]andchrysin possesses Anti-oxidant and anti-inflammatory property[36]. These compounds are reported to be found in propolis GC-MS analysis and add value to the study. Some people already started consuming Propolis as a supplementary diet and shortly the *in-vivo* studies on antidiabetic activity for propolis will fetch strong evidence for the consumption and certainly, it will be a" boom to diabetic patients".

Conclusion

The results of the present study prove that the aqueous extract of Propolis is effective against α- amylase and α- glucosidase, which are capable of maintaining postprandial glucose level. However, thekey compounds responsible for the enzyme inhibitory action ofα- amylase and α- glucosidase will be further identified andcharacterized. Hence the propolis can be added to innovate new anti-diabetic drug.

Author contribution:

Ashmitha.E and Aarthi.N carried out the experiment and contributed equally to manuscript, Aarthi.N and Harathi.P.B contributed to design and implement of the research. All authors provided important feedback to assist in research, analysis, and manuscript design.

Conflicts of Interest:

The author declare that they have no conflict of interest.

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