IN-VITRO AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF ALLIUM HUMILE AND *ORIGANUM VULGARE* FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

Diabetes mellitus is one of the major health problems in the world, the incidence and associated mortality are increasing. Inadequate regulation of the blood sugar imposes serious consequences for health. Conventional antidiabetic drugs are effective, however, also with unavoidable side effects. On the other hand, medicinal plants may act as an alternative source of antidiabetic agents. Here, the presence research investigates the *in-vitro* and phytochemical effect of ethanolic extract of *Allium humie* and *origanumvulgare*for antidiabetic activity. *Allium humie* belongs to family Alliaceae, is rich in sulfur containing compounds with distinct biological activities. The phytochemical analysis showed the presence of alkaloids, flavonoids, steroids and terpenoids.. The phytochemical test of *O.vulgare* ethanol extract was showed some compounds, such as a flavonoid, alkaloid, triterpenoid/steroid, essential oil, and tannin.*In*-vitro assays demonstrated that the extract inhibited α-glucosidase activity, promoted glucose uptake, inhibited glycosylation and relieved oxidative stress, which suggested that *O. vulgare* extract has a strong hypoglycemic capacity.

Keywords: Diabetes mellitus, medicinal plants, antidiabetic

1. INTRODUCTION

Diabetes is a persistent illness. Hyperglycemia could be one of its characteristics. These could be useful in treating both insulin action and insulin secretion abnormalities. Inadequate insulin secretion and tissue size brought on result in anomalies in the metabolism of protein, carbohydrates, and lipids. These could result in modifications or elevations in blood glucose levels. Numerous bodily systems, including blood vessels and nerves, could be harmed by them. The survey found that between 0.5 and 3 percent of people suffered from these disorders. It has increased to about 7% these days. There are between 200 and 300 million impacted people, and in the upcoming years, that number should increase or triple.[1,2]

1.1 Types of diabetes

1.1.1 Type 1 diabetes mellitus (T1DM) or Juvenile Onset Diabetes

Diabetes mellitus that is insulin dependent is another name for it (IDDM). 5 to 10 percent of the population resides there. These might result in normal glucose production and might lower the sugar level. The Langerhans islet is what this is known as. Blood glucose levels are used to regulate blood sugar levels and kill cancerous cells. The islet antibiotic cell, insulin to autoantibodies, GAD to antibodies, tyrosine phosphate, and IA-2 may all fall within this category.[5,6]

1.1.2 Adult-Onset Diabetes, also known as Type 2 Diabetes Mellitus (T2DM),

Non-insulin dependent diabetic mellitus is another name for this. Up to 98 percent of the population could be affected by this disease. This can be related to the modern style element. It was typical in adults. This might lessen the disease's symptoms. 7,8 The insulin activity may decrease as a result. It has a variety of disorders including gradual deterioration, insulin resistance, and malfunction of pancreatic beta cells. Obesity, advanced age, and a history of diabetes may all be risk factors for this condition.[9,10]

Alpine Onion Allium humile, belongs to family Alliaceae, is a species of onion found in the Himalayas, at altitudes of 3,000– 4,000 m. Leaves and inflorescences are commonly used as seasoning agents.[11]

Origanumvulgare, also called *origanum* or wild marjoram, aromatic perennial herb of the mint family (Lamiaceae) known for its flavorful dried leaves and flowering tops.[12]

2. MATERIAL AND METHODS

2.1 Collection of plant

Allium humile(AH) and *Origanumvulgare* whole plants were collected from various areas.

2.2. Preparation of Extracts

The plants of *A. humile* and *Origanumvulgare* were dried in shade and room temperature for 2 days followed by drying [40– 50 °C] for 3–4 h and powdered to obtained coarse powder. 980 gm of powder of *A.humile* and *Origanumvulgare* were extracted with ethanol and Petroleum Ether using Soxhlet extraction technique to get extracts.

The soxhlet extractor setup consists of a round bottom flask, siphon tube, distillation path, expansion adapter, condenser, cooling water inlet, cooling water outlet, heat source and thimble. This technique involves placing the powdered sample within a porous "thimble" composed of sturdy filter paper or cellulose, placing it inside the Soxhlet apparatus's thimble chamber. A heating source, such as a heating mantle, is used to warm the extraction solvent in a flask with a circular bottom. The extraction solvent used determines the heating temperature. The bottom flask's solvent vaporizes into the condenser as a result of heat, then drips back into the sample thimble. The liquid content is drained into the bottom flask once again when it reaches the syphon arm, and the clear solution in the syphon tube indicates that the operation is complete. This system's advantage may be that only one batch of heated solvent is recycled, as opposed to several parts being passed through the sample. For thermo-labile chemicals, this approach is not appropriate since prolonged heating may cause compound degradation.

With the help of heat from the distillation flask, this approach keeps the extraction temperature comparatively high. Filtration of the extract is not necessary, and bringing new solvent into contact with the solid matrix on a regular basis prevents the transfer equilibrium from being disrupted.

Once this extraction process completes the solvent was removed by evaporation under reduced pressure to obtain a semisolid mass. The resultant extracts were kept in a desiccator followed by weighing to give percentage yield of each extract.

2.3.Phytochemical Analysis

The ethanolic and Petroleum ether extracts of *Allium humile*(EEAH) and *Origanumvulgare*(EEOV) were subjected to preliminary phytochemical screening.

2.3.1. Test for Alkaloids:

The extracts were treated with dilute HCl and filtered. The filtrate was treated with various alkaloidal agents.

- Mayer's Test: Sample was treated with mayer's reagent,appearance of cream color indicated presence of alkaloids.
- Dragendorff's Test: Sample was treated with Dragendorff's reagent; appearance of reddish-brown precipitate indicated presence of Alkaloids.
- Hager's Test: Sample was treated with hager's reagent; appearance of yellow colour indicated presence of Alkaloids.
- Wager's Test: Sample was treated with wager's reagent; appearance of brown precipitate indicated presence of Alkaloids.

2.3.2. Test for carbohydrates:

The extracts were treated with 3ml of alpha naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of the two liquids indicates presence of carbohydrates.

- Fehling's Test: To the sample Fehling's solution A and B was added and heated for two minutes. Appearance of reddish-brown color indicated presence of reducing sugars.
- Benedict's test: To the sample Benedict's solution was added and heated, appearance of reddish orange precipitate indicated presence of reducing sugars.
- Barfoed's Test: The samples were treated with Barfoed's reagent and heated; appearance of reddish orange precipitate indicated presence of reducing sugars.

2.3.3. Test for Proteins:

- Biuret's Test: To the extracts copper sulphate solution followed by sodium hydroxide solution, a violet color precipitate indicated presence of proteins.
- Million's Test: To the extracts Million's reagent was added, appearance of pink color indicated presence of Proteins.

2.3.4. Test for Steroids:

- LibermannBurchard's Test: The extracts were treated with conc

  Sulphuric acid and Glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the two liquids and appearance of green color in the aqueous layer indicated presence of steroids.

2.3.5. Test for Tannins:

- The extracts were treated with 10% lead acetate solution appearance of white precipitate indicated presence of tannins.
- The extracts were treated with aqueous bromine water, appearance of white precipitate indicated presence of tannins

2.3.6. Test for Flavonoids:

5 ml of the extracts solution was hydrolyzed with 10 % Sulphuric acid and cooled. It was then extracted with diethyl ether and divided into 3 portions in three separate test tubes. 1 ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxides and 1 ml of dilute ammonia solution were added to the first second and third test tube respectively. Development of yellow color in each test tube indicated presence of flavanoids.

- Shinoda's Test: The extracts were dissolved in alcohol, to which a piece of magnesium followed by dropwise addition of ConcHCl and heated. Appearance of Magenta color indicated presence of flavanoids.

2.3.7. Tests for Gums and mucilage:

The extracts were treated with 25 ml of absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

2.3.8. Test for Glycosides:

A pinch of the extracts were dissolved in glacial acetic acid and a few drops of ferric chloride solution was added followed by the addition of Conc.Sulphuric acid, formation of red ring at the junction of the two liquids indicated presence of glycosides.

2.3.9. Test for Saponins: Foam Test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tube indicated presence of saponins.

2.4 Test for Terpenes:

The extracts were treated with tin and thionyl chloride, appearance of pink colour indicated presence of terpenes.

2.5. Test for Total polyphenolic content:

Total polyphenolic content was determined according to the European Pharmacopoeia, using the Folin–Ciocalteu method, two milliliters of extract were diluted 25 times, then mixed with 1.0 mL of Folin–Ciocalteu reagent, 10.0 mL of distilled water and diluted to 25.0 mL with a sodium carbonate solution (290 g/L). Absorbance was measured at 760 nm, after 30 min.

2.6 *IN-VITRO* ANTIOXIDANT ACTIVITY

2.6.1 DPPH radical scavenging assay

Free radical scavenging ability of the drugs was tested by DPPH radical scavenging assay as described by Rahman et al., 2015. The hydrogen atom donating ability of the drugs was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produced purple/violet color in methanol solution and fades in presence of antioxidants to shades of yellow color. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 ml of this solution was mixed with 1.6 ml of extract in methanol at varied concentrations of 12.5 to 150 μg/ml. The solution mixture was mixed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. Butylated hydroxytoluene (BHT) was used as a reference. Percentage of DPPH radical scavenging activity was calculated by the following equation:

%DPPH radical scavenging activity = $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the drugs/standard. Then % of inhibition was plotted against concentration. The experiment was repeated three times at each concentration.

2.6.2 Total antioxidant capacity (ABTS assay)

This assay is based on decolorization that occurs when the radical cation ABTS is reduced to ABTS'(2, 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid). In brief, the radical was generated by reaction of a 7 mM solution of ABTS in water with 2.45 mM potassium persulphate ($K_2O_8S_2$) (1:1). The mixture was held in darkness at 27°C for 16 h (time needed to obtain stable absorbance at 734 nm). After incubation, the radical solution was further diluted with water (1 mL of ABTS reagent + 27 mL DW) until the initial absorbance value of 0.7 ± 0.005 at 734 nm was reached. For the assay of test samples 980 µl of ABTS reagent was mixed with 20 µL of the sample or standard. Absorbance was taken after 6 min at 734 nm. ΔO.D. was calculated between initial (0 min.) and 6^{th} min. reading. As a standard, ascorbic acid (8.8 µg/mL to 88.0 µg/mL). The percentage of scavenging inhibition capacity of ABTS of the extract was calculated using the following equation and compared with ascorbic acid.

% inhibition = $[(Abs_{control} - Abs_{sample})]/ (Abs_{control})] \times 100$

3. RESULTS

3.1 Phytochemical Analysis

The presence of various phytochemical constituents in the extract was determined using standard screening tests.

S.No	Ingredients	EEAH	PEAH	EEOV	PEOV
1	Alkaloids	++	+	+	+
2	Amino Acids	+	+	+	+
3	Flavonoids	++	+	-	+
4	Glycosides	-	-	-	+
5	Gums and Mucilage	-	-	+	-
6	Proteins	+	++	+	+
7	Carbohydrates	++	++	+	+
8	Steroids	++	++	+	+
9	Tannins	+	+	+++	++
10	Terpenoids and Saponins	+	+	+++	++
11	Polyphenolic compounds	+	+	+++	+++

+ present, -absent

3.2 *IN-VITRO* ANTIOXIDANT ACTIVITY

3.2.1 DPPH radical scavenging assay

The results indicate that EEAH and EEOVinhibited free radical generation based on concentrations and assays used. The scavenging activity of DPPH·increased proportionally with extract concentration from 0 to 150 µg/mL. The results revealed that at the maximum concentration (150 µg/mL), EEAH and EEOV possessed the highest scavenging activity (71% and 79%).

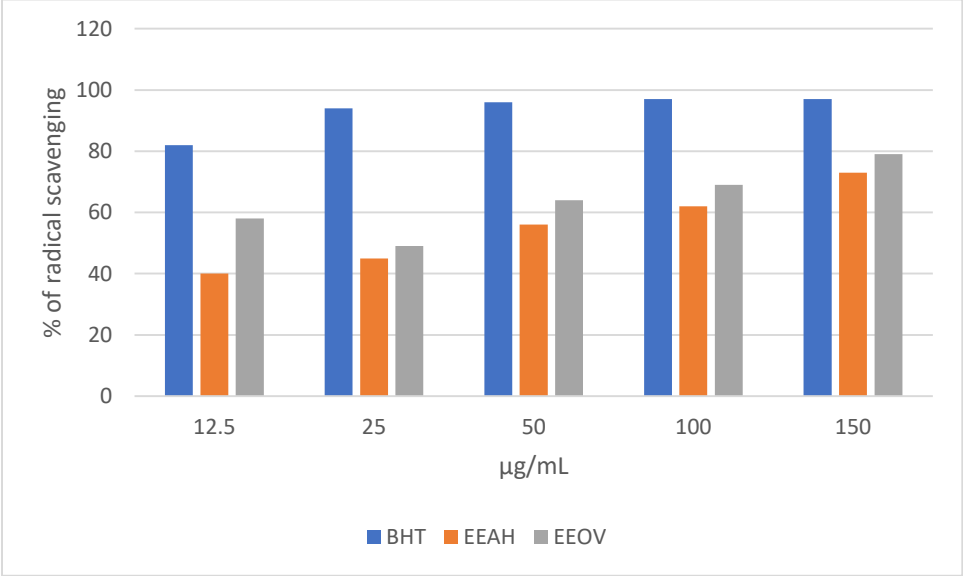

Figure 1: Determination of DPPH radical scavenging activity of EEAH and EEOV.

3.2.2 ABTS radical scavenging assay (EEAH and EEOV)

The results of ABTS method indicated that EEAH and EEOV decreased the generation of ABTS free radical, which was likely to be related to the scavenging capacity of extracts and standard compound (ascorbic acid). The ABTS radical inhibition efficacies of EEAH and EEOV was found to be lower than that of standard compound at all concentrations (0 – 150 µg/mL). The results revealed that at the maximum concentration (150 µg/mL), EEAH and EEOV possessed the highest scavenging activity (67% and 71%).

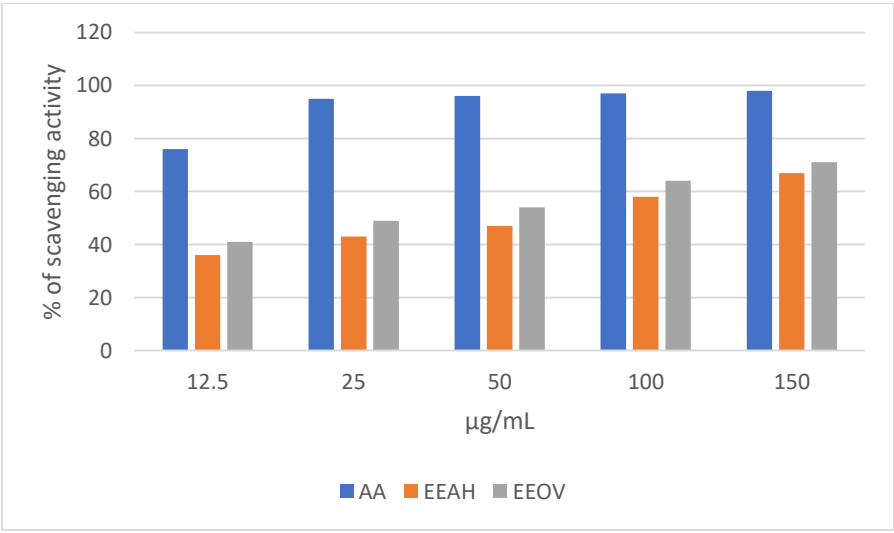

Figure 2: Determination of ABTS radical scavenging activity of EEAH and EEOV.

4. CONCLUSION

We can conclude that *Allium humile*(AH) and *Origanumvulgare*is a rich source of natural antidiabetic agents. DPPH and ABTS study showed potential inhibitions in all tested *in-vitro* antidiabetic targets. Further, both the compounds were also able to decrease the blood glucose levels in experimental animals.

5. REFERENCES

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