Research Article

Enzymes Inhibition and Antioxidant Potential of Medicinal Plants Growing in Oman

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The recent study was designed to explore Dodonaea viscosa, Juniperus excelsa, Helianthemum lippii, and Euryops pinifolius using methanolic (MeOH) extract. Their subfractions were examined against urease, carbonic anhydrase II (CA-II), α-glucosidase enzymes, and free radicals scavenging significance based on local practices via standard methods. Significance potential against the urease enzyme was presented by ethyl acetate fraction (EtOAc) of D. viscosa with (IC₅₀ = 125 ± 1.75 μg/mL), whereas the H. lippii (IC₅₀ = 146 ± 1.39 μg/mL) in the EtOAc was found efficient to scavenge the free radicals. Besides, that appreciable capacity was observed by the J. excelsa, D. viscosa, J. excelsa, and E. pinifolius as compared to the standard acarbose (IC₅₀ = 377 ± 1.24 μg/mL). Maximum significance was noticed in methanolic (MeOH) extract of J. excelsa and presented carbonic anhydrase CA-II (IC₅₀ = 5.1 ± 0.20 μg/mL) inhibition as compared to the standard (acetazolamide). We are reporting, for the first time, the CA-II inhibition of all the selected medicinal plants and α-glucosidase, urease, and antioxidant activities of the E. pinifolius. Thus, further screening is needed to isolate the promising bioactive ingredients which act as an alternative remedy to scavenge the free radicals, antiulcer, and act as a potential source to develop new antidiabetic drugs for controlling postprandial blood sugar as well as carbonic anhydrase inhibitors.

1. Introduction

Phytomedicines are made up of medicinal plants and their chemical ingredients and have a key therapeutic role in various health-related complications, for instance, gastrointestinal infections, free radicals scavenging, and antidiabetic properties [1]. In this context, plant extracts are made up of a variety of chemical elements and are well-known for their wide range of clinical applications. They are derived from plants using both traditional and other modern approaches [2].

Urease enzymes play a leading role to catalyze the hydrolysis of urea, thus gaining substantial attention regarding human health and their life qualities [3–5]. It maintains optimum pH and treats the NH₃ to balance its medium level due to which they own an incredible medical position [6, 7]. It is the main public health matter related to the bacterium H. pylori, which can endure in an acidic environment of the stomach having pH 2 range [6, 8]. The high prevalence of H. pylori in the human population indicates that such microbes have developed mechanisms for resistance against host defenses [8]. Marketed available urease drugs (phosphorodiamidate, hydroxamic acid derivatives, and imidazoles) are much more toxic with less efficacy rate and thus influenced by their limited clinical use [9, 10]. Thus, the quest for innovative urease inhibitors with enhanced stability and minimal toxicity is needed to improve the life quality of human beings and animals. Therefore, plant-based drugs are the alternative basis for having the ability to overcome the mentioned complications.

Diabetes mellitus (DM) is a common metabolic illness that has become a serious worldwide health issue. When DM is left untreated, it can harm the nerves, eyes, kidneys, and other organs. Increased urination, impaired vision,
weariness, hunger, and thirst are among the symptoms of T2DM [11]. Controlling postprandial hyperglycemia by delaying carbohydrate digestion and absorption is one of the therapy options for T2DM. α-Glucosidase (EC 3.2.1.20) is an enzyme found on the small intestine’s brush edge. Inhibition of the α-glucosidase can limit the digestion of carbohydrates resulting in declined postprandial blood sugar levels [12]. As a result, α-glucosidase inhibitors (AGIs) can be used as first-line therapy for T2DM [13–15].

Natural antioxidants can improve food quality including color, taste, flavor, and stability and also act as standardized nutrients (nutraceuticals) to end up the attack of free radicals in biological systems [16, 17], that might deliver additional health benefits to consumers [18, 19] and reduce the risk of disorders caused by free radicals [20]. Recently, considerable attention focused on the use of natural antioxidants to defend the human body against brain tissues and neurological disorders associated with free radical damage [20, 21]. This research is focused on searching for new sources of natural antioxidants and urease inhibitors that can be used directly or in combination with other official drugs as a lead compound for drug discovery.

Carbonic anhydrase (CA) is a metalloenzyme that contains zinc and is mainly used to catalyze CO2 hydration into bicarbonate and hydrogen ions [22]. The CA inhibitors control the enzymatic actions and prevent bicarbonate reabsorption which leads to numerous adverse effects such as potassium and bicarbonate retention in the human urine and decreased sodium absorption as a diuretic [23]. The intake of synthetic drugs for longer use to release this complication might be harmful. Therefore, searching for plant-based alternative remedies can be useful to cope with these disorders [24].

*Juniperus excelsa* M. Bieb (JE, Cupressaceae) is used mainly for lowering blood pressure [25], jaundice, bronchitis, tuberculosis, common cold [26], diabetes, stomachache, grazing, and wood harvesting [27]. Literature surveys documented its positive effects in treating colds, cough, dysmenorrhea, persuading menses, and expelling fetuses [28, 29]. *Dodonaea viscosa* Linn (DV, Sapindaceae) was reported to possess antiviral, anti-inflammatory, laxative, spasmylytic, antimicrobial, hypotensive agents [30], smooth muscle relaxant, anesthetic, throat infection, malaria, and antiulcerogenic [31, 32]. Traditionally, it is used to treat many illnesses like malaria, cold, aches, fever, toothaches, rheumatism, headaches, ulcers, diarrhea, dysmenorrhea, irregular menstruation, and constipation [33].

*Helianthemum lippii* (HL) belongs to the genus *Helianthemum*, which is a widely distributed and most taxonomically complex genus of the Family Cistaceae. Alsabri et al. [34] reported anti-inflammatory and analgesic activity of *H. lippii* against carrageenan-induced paw edema and hotplate-induced pain in rats. Previous studies showed that the plant is a rich source of polyphenols and flavonoids [35, 36] with antioxidant, antiulcer, and antimicrobial [37], as well as cytotoxic [38]. *Euryops pinifolius* A. Rich belongs to the genus *Euryops* (family: Asteraceae) [39], mostly cultivated in southern Africa, with a few species in other parts of Africa and on the Arabian Peninsula [28]. The local uses of *E. pinifolius* are least known, however, in some places of the Arabian Peninsula (Yemen, Oman, and Saudi Arabia), are used to wound healing [40].

To devise innovative plant-derived drugs, four important medicinal plants were collected from Al Jabal Al Akhdar (Northern Oman) and evaluated for antioxidant and enzyme inhibition activities. To the best knowledge, this is the first report on the enzyme inhibition study of these plants. In addition, we are also reporting the antioxidant activity of *H. lippii* and *E. pinifolius* for the first time.

### 2. Materials and Methods

#### 2.1. Collection and Identification of the Medicinal Plants.

Aerial parts of the plant species, viz., *J. excelsa* (3.5 Kg), *H. lippii* (3.5 Kg), *E. pinifolius* (4.0 Kg), and *D. viscosa* (5.5 Kg), were collected from Al Jabal Al Akhdar, Oman, identified by a plant taxonomist (Dr. Syed Abdullah Gillani) at the Department of Biological Sciences and Chemistry, University of Nizwa, Oman. After documentation, voucher specimens (HL-01/2012, EP-02/2012, DV-03/2012, and JE-04/2012) were kept at the herbarium of Natural and Medical Sciences Research Center, University of Nizwa, Oman, for further processing.

#### 2.2. Extraction and Fractionation.

The whole aerial parts of the *Dodonaea viscosa* were dried, chopped, and soaked in methanol at room temperature for 15 days three times as reported earlier by Shah et al. [16]. Evaporation of the MeOH in vacuo at 45°C yielded a crude methanol extract, which after suspension in water was successively fractionated into n-hexane, dichloromethane (CH2Cl2), ethyl acetate (EtOAc), butanol (n-BuOH), and aqueous (H2O) (Figure 1). The same procedure was used for the extraction and fractionation of other medicinal plants. The details of quantity of crude extracts and the different fractions of the selected plants are given in Table 1. The crude extracts and their fractions were subjected to biological screening to determine their potential effect.

#### 2.3. Antioxidant Activity.

The antioxidant bioassay of the crude extracts and subfractions of the selected plants was evaluated using α, α-diphenyl-β-picrylhydrazyl (DPPH) assay [41]. About 100 μL of methanol was mixed with 150 μL of DPPH solution as a negative control. For the sample, about 150 μL of DPPH was added with 100 μL of three concentrations of extract (100, 500, and 1000 μg/mL). The absorbance was taken at 517 nm by spectrophotometer. All the results were compared to a control containing 50 μL of methanol. The positive control used was ascorbic acid (Table 1). Each test was repeated three times, and % inhibition was calculated as:

\[
\text{Percent (\%)} \text{ inhibition of DPPH activity : } A_c - A_s / A_c \times 100 ,
\]

\[
A_c = \text{Absorbance of control (ascorbic acid) ;}
\]

\[
A_s = \text{Absorbance of sample.}
\]

#### 2.4. Urease Enzyme Inhibition.

Urease enzyme inhibition
About 25 μL solution of Jack bean urease was mixed with 50 μL urea dissolved in phosphate buffer (pH 8.20) with 20 μL of three concentrations (100, 500, and 1000 μg/mL) of different extract fractions and then incubated at 37 °C for 15 min. Then, 50 μL of solution B (phenol reagent (1% w/w phenol) own expect 3 mg in 30 mL + 0.005% w/v sodium nitroprusside) and 70 μL of solution A (alkali reagent (0.5% w/v NaOH +1% active chloride NaOCl)) were added and then incubated again for 15 min. Thiourea was used as a positive (standard) control, while methanol was used as a negative control (Table 2). The absorbance was recorded at 630 nm with a total volume of 215 μL.

\[
\text{OD}_{\text{test compound}} = \text{Optical Density of control (thiourea)}; \\
\text{OD}_{\text{control}} = \text{Optical Density of the sample}. 
\]  

2.5. α-Glucosidase Assay. All the twenty-four samples of crude extract and subfractions were evaluated in vitro against α-glucosidase enzyme (E.C.3.2.1.20) as described earlier by Shah et al. [11], by using (50 mM) phosphate buffer of pH (6.8). The enzyme was properly dissolved in the phosphate buffer; 1 U/2 mL, 20 μL/well of the enzyme, and 135 μL/well phosphate buffer was used as reaction buffer, 20 μL/well of the tested samples were solubilized in DMSO (0.5 μg/mL), in 96-wells plates incubated for 15 min at 37 °C. After the incubation period, the substrate para nitro phenyl-D-glucopyranoside was added at a concentration of 0.7 mM, and the change in absorbance was measured at 400 nm for 30 minutes. The positive control used was acarbose, and DMSO was used as negative control.

\[
\text{OD}_{\text{test compound}} = \text{Optical Density of control (acarbose)}; \\
\text{OD}_{\text{control}} = \text{Optical Density of the sample}. 
\]  

2.6. Carbonic Anhydrase II Inhibition Assay. The total reaction mixture comprised 20 μL (0.5 mmol/well) of test compounds (10% DMSO in total), and then add HEPES-Tris buffer 140 μL (20 mmol, pH =7.4), 20 μL of purified bovine erythrocyte CA-II (1 mg/mL, 0.1 units/well) prepared in buffer, and finally substrate 4-nitrophenyl acetate (4-NPA, 0.7 mmol) 20 μL to attain final volume 200 μL/well [44, 45]. An enzyme (EC 4.2.1.1, Sigma-Aldrich, St. Louis, MO, USA) along with the tested compounds was incubated for 15 min in a 96-well plate. Then, the reaction was started with the addition of 20 μL of the substrate (4-nitrophenyl acetate) and continuously monitored the rate (velocities) of product formation for 30 min with the intervals of 1 min, at 25°C by using a microplate reader (Bio-Rad, Molecular Devices, CA, USA). Acetazolamide and DMSO were used as positive and negative controls, respectively.

\[
\text{OD}_{\text{test compound}} = \text{Optical Density of control (acetazolamide)}; \\
\text{OD}_{\text{control}} = \text{Optical Density of the sample}. 
\]  

2.7. Statistical Analysis. The SoftMax Pro package and Excel were utilized.
3. Result and Discussion

Herbs have been used as a source of medicine since the dawn of human civilization, and they continue to play an important role in clinical use and quality control for a variety of health issues [46].

3.1. Antioxidant Capability. The antioxidant significance of the selected plants is determined to evaluate the free radical scavenger capacities of the selected plant species utilizing ascorbic acid as a standard inhibitor as presented in Table 2. In recent years, increasing attention has been paid to antioxidant compounds (flavones, anthocyanin, flavonoids, catechin, isoflavones, and other phenolics) derived from plants due to their valuable role in reducing various disorders such as immune system, brain dysfunction, heart disease, decline, cardiovascular disease, aging, and cancer [47]. The free radicals produced due to human metabolism affect the cellular membrane to overcome these complications [47]. The investigation reveals that among the screened four plant species, *H. lippii* fractions offered a significant ability to scavenge the free radicals and act as an antioxidant agent. The EtOAc fraction of *H. lippii* exhibited the highest potential to act as an antioxidant agent with IC$_{50}$ of 146 ± 1.39 μg/mL followed by the MeOH (IC$_{50}$ = 368 ± 2.18 μg/mL) and aqueous extract (460 ± 1.21 μg/mL), respectively. This significance is attributed due to the presence of an affluent basis of polyphenolic constituents as documented by Benabdelaiz et al. [48]. Alali et al. [49] investigated *H. lippii* from Jordan and reported methanol (IC$_{50}$ = 176.1 μmol TE g$^{-1}$dry weight) and aqueous (IC$_{50}$ = 176.1 and 274.2 μmol TE g$^{-1}$dry weight) extracts in comparison to the standard via ABTS assay. However, in the current study, the geographical location, collection, habitat, harvesting season, screening approach, and standard used are different from earlier studies. Therefore, our finding reveals that the *H. lippii* has a significant ability to neutralize the free radicals. Belyagoubi et al. [36] collected *H. lippii* from Algeria as a plant habitat influenced the quality and quantity of bioactive compounds responsible for promising pharmacological potentials [50]. However, moderate capability was observed in the *n*-hexane and CH$_2$Cl$_2$ fractions (Table 2). In the case of *E. pinifolius*, the EtOAc fraction displayed significance inhibition (IC$_{50}$ = 378 ± 1.56 μg/mL) followed by the n-BuOH fraction (IC$_{50}$ = 386 ± 1.21 μg/mL). Moreover, the EtOAc fraction of *D. vicia* also produced promising findings with (IC$_{50}$ = 386 ± 1.65 μg/mL) ensued by the n-BuOH fraction (IC$_{50}$ = 467 ± 1.84 μg/mL), while normal activity was examined by the MeOH extract (Table 2). The current findings consented to the data stated for some Yemeni traditional medicinal plants by Mothana et al. [51] that *D. vicia* was one of the most active plants that showed promising antioxidant activity. In addition to that, the current outcome also supports the results reported by Singh et al. [52] for *Rhus aucheri* as the understudy plant was collected from Oman. Recently, Muhammad et al. [53] isolated some flavonoids from the EtOAc fraction of *D. vicia* showed higher antioxidant activity further stringent our findings. It was also observed that the free radicals scavenging significance of

| Plant Species | Fractions | Antioxidant activity IC$_{50}$ ± S.E.M (μg/mL) |
|---------------|-----------|---------------------------------------------|
| *J. excelsa*  | n-Hexane  | Nd                                          |
|               | DCM       | Nd                                          |
|               | EtOAc     | 402 ± 2.15                                  |
|               | n-BuOH    | 428 ± 1.54                                  |
|               | MeOH      | Nd                                          |
|               | Aqueous   | Nd                                          |
|               | n-Hexane  | 788 ± 2.68                                  |
|               | DCM       | 658 ± 1.54                                  |
| *E. pinifolius* | EtOAc     | 378 ± 1.56                                  |
|               | n-BuOH    | 904 ± 2.68                                  |
|               | MeOH      | Nd                                          |
|               | Aqueous   | Nd                                          |
| *H. lippii*   | EtOAc     | 146 ± 1.39                                  |
|               | n-BuOH    | 561 ± 1.34                                  |
|               | MeOH      | 368 ± 2.18                                  |
|               | Aqueous   | 460 ± 1.21                                  |
|               | n-Hexane  | Nd                                          |
|               | DCM       | Nd                                          |
| *D. vicia*    | EtOAc     | 386 ± 1.65                                  |
|               | n-BuOH    | 467 ± 1.84                                  |
|               | MeOH      | 914 ± 2.61                                  |
|               | Aqueous   | Nd                                          |

Ascorbic acid = 6.25 ± 0.56 μM; DCM: dichloromethane; EtOAc: ethyl acetate; BuOH: n-butanol; MeOH: methanol; SEM: standard error mean; Nd: not determined (Conc. = 1 mg/mL).

The given formula below was used to calculate percent inhibition.

\[
\text{%Inhibition} = 100 - \left( \frac{O.D_{\text{test compound}}}{O.D_{\text{control}}} \right) \times 100. \quad (5)
\]

EZ-FIT (Perrella Scientific, Inc., USA) was used for IC$_{50}$ calculations of all tested samples. To overcome on the expected errors, all experiments were performed in triplicate, and variations in the results are reported in standard error of mean values (SEM).

\[
\text{SE} = \frac{\sigma}{\sqrt{n}}. \quad (6)
\]
some medicinal plants from Iran was dissimilar from our
recorded data as described by Boroomand et al. [54] due to
the variation of their habitat, climatic, topographic, and
edaphic factors influenced the content and quality of the
metabolites accountable to act as an antioxidant agent. The
data obtained from these in vitro models demonstrated the
strong antioxidant potential of EtOAc and n-BuOH frac-
tions of the selected medicinal plants, which might be a con-
cern with its high medicinal and pharmaceutical use as a
functional food in the treatment of different diseases.

3.2. Antiulcer Potential. The urease enzyme inhibitory activ-
ity of crude extracts/fractions of the plants was determined
using a concentration of 1.0 mg/mL. Ethyl acetate fraction of
D. viscosa exhibited significantly promising urease inhibi-
tion (IC\textsubscript{50} = 125 ± 1.75 μg/mL), followed by n-hexane
(IC\textsubscript{50} = 142 ± 2.00 μg/mL) and n-BuOH (IC\textsubscript{50} = 410 ± 2.50 μg/mL) fractions. The data of crude extract and fractions of J. excelsa revealed that only the EtOAc fraction exhibited significant inhibition (IC\textsubscript{50} = 173 ± 2.50 μg/mL) as compared to other fractions. In the case of H. lippii, the EtOAc fraction showed significantly strong inhibition (IC\textsubscript{50} = 257 ± 1.25 μg/mL), followed by n-BuOH (IC\textsubscript{50} = 435 ± 2.75 μg/mL), while MeOH and aqueous frac-
tions of the same plant did not show activity (Table 3).

The EtOAc fraction of E. pinifolius attributed promising
inhibition (IC\textsubscript{50} = 730 ± 2.50 μg/mL), followed by the n-
BuOH (IC\textsubscript{50} = 430 ± 2.25 μg/mL), while other fractions
did not show urease inhibition (Table 3).

These findings provide crucial information about the
biologically active constituents present in medicinal plants
truly responsible for the inhibition of the urease enzyme.
Thus, our findings matched with the data reported by Rauf
et al. [55] for Diospyros lotus roots and in favor of the out-
comes presented by Maherina et al. [56] as the use of the
same approach to determine the urease significance in the
medicinal plants. Moreover, our current findings do not
agree with the data reported by Tahseen et al. [57] due to
their variability in their habitat. In the future, bioassay-
guided isolation of these secondary metabolites might be
exciting and interesting to know the chemical constituents
responsible for inhibition and to understand their basic
mechanism against these enzymes.

3.3. Antidiabetic Significance. Crude extract and subfractions
of the four plant species (D. viscosa, J. excelsa, H. lippii, and
E. pinifolius) were tested to analyze their antidiabetic poten-
tial by targeting the key carbohydrate digestive enzyme α-
glucosidase. Furthermore, the aqueous and n-hexane frac-
tions of J. excelsa showed below 50% inhibitory activity
and were found to be inactive. While other samples dis-
played several folds of potent inhibitory potential in the
range of 1.30-20.75 μg/mL, compared with acarbose
(IC\textsubscript{50} = 377.24 ± 1.14 μg/mL). Moreover, the n-BuOH and
n-hexane fractions of D. viscosa exhibited significant inhibi-
try activity with IC\textsubscript{50} (1.30 ± 0.05 and 2.04 ± 0.06 μg/mL),
respectively. Thus, our data is supported by the literature
explained by Assefa et al. [58] and VVM et al. [59]. It might
be due to the presence of active chemical ingredients having
the ability to cure diabetes. On the other hand, the EtOAc,
DCM, aqueous, MeOH, n-hexane, and n-BuOH fractions
of J. excelsa exhibited potent α-glucosidase inhibitory poten-
tial with IC\textsubscript{50} (1.31 ± 0.02, 3.65 ± 0.12, 2.48 ± 0.13, 3.11 ±
0.14, 2.78 ± 0.11, and 2.05 ± 0.08 μg/mL), respectively. Thus,
our current screening consented to the findings of Bhatia
et al. [60], which depicted a little variation in outcomes
reported by Sancheti et al. [61] and Gok et al. [62]. Due to
differences in the chemical ingredients influenced by envi-
ronmental factors and the solvents and methods used in
our studies, in addition to that, the E. pinifolius offered var-
iations in the anti-α-glucosidase potential. For instance,
the MeOH extract was found to be the most potent and dis-
played IC\textsubscript{50} = 2.86 ± 0.03 μg/mL. A slight decrease in the
α-glucosidase inhibitory activity was observed in the other
fraction samples, such as EtOAc having IC\textsubscript{50} = 7.85 ± 0.16
μg/mL. Likewise, a slightly further decline in the antidiabetic
capability was observed, in the DCM and n-BuOH fractions
depicted (IC\textsubscript{50} = 16.72 ± 0.15 μg/mL and 22.12 ± 0.15 μg/
ML), respectively. So, these outcomes also reflect that our
data agrees with the findings of Khatib et al. [63].

Furthermore, our investigation exhibited a little varia-
tion as compared to the data stated by Ibrahim et al. [64]
as mentioned previously that the habitat variation can also
be led to variability among the chemical ingredients among
the different and same plant species. Interestingly all the
fractions of H. lippii displayed several fold potent inhibitory
activities with almost the same potency comprises of EtOAc,
DCM, aqueous, n-hexane, and BuOH with IC\textsubscript{50} of 5.12 ±
0.18, 5.73 ± 0.21, 5.47 ± 0.13, 5.73 ± 0.21, and 6.45 ± 0.11
μg/mL, respectively, as compared to MeOH extract
(IC\textsubscript{50} = 10.48 ± 0.26 μg/mL). Our current study consented
to the literature described by Zarei et al. [65]. However,
our current result does not agree with the findings of Run-
prom et al. [66]. The similarity of the antidiabetic signifi-
cance presented by the medicinal plants might be due to
the presence of the phenols and flavonoids. As we know in
the current era plant extractions are becoming increasingly
popular in medicinal therapies, and they are an alternative
and valuable herbal medicinal medicine because of their
broad usage and lower adverse e
ffects, the current results
insight into the crucial therapeutical importance of the D. vis-
cosa, J. excelsa, H. lippii, and E. pinifolius in their crude and
subfractions. Hence, these promising findings might be used
as a therapeutic approach for the management of type 2 dia-
betes (T2DM) displayed in Table 3.

3.4. Carbonic Anhydrase II Significance. The selected plants
are profiled for their carbonic anhydrase activity as shown
in Table 3. Among the subfractions, EtOAc fraction of the
D. viscosa presented significant activity with IC\textsubscript{50} of 27.5
± 3.12 ± μg/mL trail by the MeOH extract IC\textsubscript{50} = 50.4 ±
2.03 μg/mL, while other subfractions were found inactive.
The D. viscosa contains phenols and polyphenols having the
ability to act as carbonic anhydrase inhibitors; thus,
our findings agree with the data stated by Karioti et al.
[67]. The current finding presented that our data do not
match with the data recorded by Rudenko et al. [68] because
environmental stress influences the quality and quantity of
bioactive ingredients responsible for numerous biological activities. Furthermore, the MeOH extract of *J. excelsa* followed by the EtOAc fraction of *J. excelsa* displayed significant potential an IC\(_{50}\) = 5.1 ± 0.20 and IC\(_{50}\) = 38.4 ± 2.52 \(\mu\)g/mL significance in comparison to other tested fractions. The n-BuOH fraction of *E. pinifolius* presented appreciable significance having IC\(_{50}\) = 41.5 ± 0.82 \(\mu\)g/mL, followed by the DCM fraction with IC\(_{50}\) = 45.4 ± 2.08, and the EtOAc fraction exhibited an IC\(_{50}\) = 47.0 ± 3.99 \(\mu\)g/mL potential, whereas the MeOH and n-hexane extract were found inactive for carbonic anhydrase activity. The *H. lippii* fractions also displayed appreciable potential except for the DCM and MeOH extracts, while aqueous extract was most potent presented IC\(_{50}\) = 9.9 ± 0.35 \(\mu\)g/mL, proceeded by the n-hexane with IC\(_{50}\) = 18.8 ± 3.13 \(\mu\)g/mL and IC\(_{50}\) = 35.6 ± 1.32 \(\mu\)g/mL in comparison to the standard acetazolamide having an IC\(_{50}\) = 4.04 ± 1.63 \(\mu\)g/mL. The current results also match up with the outcomes of Aydin et al. [69] for *Satureja cuneifolia* and dodoneine by Carreyre et al. [70] which was found effective for the carbonic anhydrase activity. However, the study reported for the bioactive ingredient dodoneine by Carreyre et al. [70] was significant as compared to our current findings because might be bioactive compounds are responsible compounds as compared to our selected plants and tested fractions.

### 4. Conclusion

In conclusion, the selected medicinal plants (D. viscosa, J. excelsa, H. lippii, and E. pinifolius) possess significance anti-ulcer, antioxidant, anti-diabetic and carbonic anhydrase-II inhibition and can be considered as essential source of bioactive ingredients. Additionally, up to now, no such scientific data were reported for the enzyme inhibition potential, whereas the two plant species were reported for the first time in a recent study. Therefore, it could be

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**Table 3:** \(\alpha\)-Glucosidase CA-II and urease activities of the selected medicinal plants.

| Sample code | Fractions | \(\alpha\)-Glucosidase IC\(_{50}\) ± SEM (\(\mu\)g/mL) | Urease IC\(_{50}\) ± SEM (\(\mu\)g/mL) | CA-II IC\(_{50}\) ± SEM (\(\mu\)g/mL) |
|-------------|-----------|---------------------------------|---------------------------------|---------------------------------|
| D. viscosa  | EtOAc     | 5.99 ± 0.20                     | 125 ± 1.75                      | 27.5 ± 3.12                     |
|             | DCM       | 10.86 ± 0.17                    | 416 ± 1.50                      | NA                              |
|             | Aqueous   | 5.34 ± 0.14                     | NA                              | NA                              |
|             | MeOH      | 3.18 ± 0.10                     | NA                              | NA                              |
|             | n-Hexane  | 2.04 ± 0.06                     | 142 ± 2.00                      | NA                              |
|             | n-BuOH    | 1.30 ± 0.05                     | 410 ± 2.50                      | NA                              |
|             | EtOAc     | 1.31 ± 0.02                     | 173 ± 2.50                      | 38.4 ± 2.52                     |
|             | DCM       | 3.65 ± 0.12                     | NA                              | 46.3 ± 1.95                     |
| J. excelsa  | Aqueous   | 2.48 ± 0.13                     | NA                              | 51.3 ± 1.35                     |
|             | MeOH      | 3.11 ± 0.14                     | NA                              | 5.1 ± 0.20                      |
|             | n-Hexane  | 2.78 ± 0.11                     | NA                              | NA                              |
|             | n-BuOH    | 2.05 ± 0.08                     | NA                              | 66.8 ± 3.19                     |
|             | EtOAc     | 7.85 ± 0.16                     | 390 ± 2.50                      | 47.0 ± 3.99                     |
|             | DCM       | 16.72 ± 0.15                    | NA                              | 45.4 ± 2.08                     |
| E. pinifolius| Aqueous  | N/A                             | NA                              | 98.2 ± 4.84                     |
|             | MeOH      | 2.86 ± 0.03                     | NA                              | NA                              |
|             | n-Hexane  | N/A                             | NA                              | NA                              |
|             | n-BuOH    | 22.12 ± 0.15                    | 430 ± 2.25                      | 41.5 ± 0.82                     |
|             | EtOAc     | 5.12 ± 0.18                     | 257 ± 1.25                      | 35.6 ± 1.32                     |
|             | DCM       | 5.73 ± 0.21                     | NA                              | NA                              |
| H. lippii   | Aqueous   | 5.47 ± 0.13                     | NA                              | 9.9 ± 0.35                      |
|             | MeOH      | 10.48 ± 0.26                    | NA                              | NA                              |
|             | n-Hexane  | 5.73 ± 0.21                     | NA                              | 18.8 ± 3.13                     |
|             | n-BuOH    | 6.45 ± 0.11                     | 435 ± 2.75                      | 59.4 ± 2.33                     |
| Acarbose    |           | 608.21 ± 1.74                   | NA                              | 1.58 ± 0.95                     |
| Thiourea    |           |                                  | 4.04 ± 1.63                     |

DCM: dichloromethane; EtOAc: ethyl acetate; BuOH: butanol; MeOH: methanol; N/A (nonactive); concentration =0.5 mg/mL; SEM: standard error mean; ND: not determined.
contended that the medicinal plants have significant potential to serve as an antioxidant and own enzyme inhibitory attributes. However, further investigations are considered necessary for the isolation and identification of the chemical ingredients accountable for the antioxidant and enzymatic significance of the selected plants.

Data Availability
All datasets on which the conclusion of the manuscript relies are presented in the paper.

Conflicts of Interest
The authors declare that they have no conflict of interest regarding this manuscript.

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