DETERMINATION OF BIOACTIVE POTENTIAL OF EVOLVULUS ALSINOIDES AND TODDALIA ASIATICA ROOT EXTRACTS

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KEYWORDS

Evolvulusalsinoides, Toddalia asiatica, anti-bacterial, anti-oxidant, anti-inflammatory and anti-diabetic properties.

ABSTRACT:

Evolvulusalsinoides, locally known as Vishnukranthi and *Toddalia asiatica,* commonly called as Kindu mullu which are well-known medicinal plants noted for itsmiraculousheathcare benefits. The present study focused on the determination of bioactive potential include anti-bacterial, anti-oxidant, anti-inflammatory and anti-diabetic activities of selected two plants. In the Disc diffusion method, the plant samples explored varying degrees of antibacterial activity against gram-positive bacteria(GPB) including *Bacillus subtilis* and *Staphylococcus aureus*, as well as gram-negative bacteria (GNB) including *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas fluorescens*. The higher DPPH, $H_2O_2$and NO scavenging activities were found in both the plant samples. From the results of anti-diabetic studies, we found that *E.alsinoides* and *T.asiatica* showed the excellent α-amylase and α-glucosidase inhibitory effect. This study conclude that *E.alsinoides* and *T.asiatica*are the rich source of bioactive potential with anti-bacterial, anti-oxidant, anti-inflammatory and anti-diabetic properties.

INTRODUCTION

Curatives made from plants are becoming more popular for treating a variety of illness and thus the pharmaceutical businesses have improved the status of therapeutic plants. The majority of conventional medications used in various therapeutics are from plants and its pharmacological activities are traced to their secondary metabolites. India is renowned for its valuable herbal medicinal knowledge legacy, biodiversity and tribal communities with in-depth knowledge of the traditional medical system (Sharma et al., 2013). Typical criteria for drug evaluation involve the pharmaceutical product's and bulk drug's understood therapeutic value, recognition studies, purity, content, consistency, chemical and physical stability, and biological availability. In accordance with the new phytopharmaceuticals rule, current formulation development, fractionation, solvent extraction and other advanced drug development methods are all permitted (Bhatt et al., 2016) Impact of free radicals on biology, toxicology, human disease, and food deterioration have received an extensive amount research investigation.

Any chemical organization that has a minimum of one unpaired electron and is present solely in an orbital is deemed a free radical. Though their reactivities differ, radicals are typically less stable than non-radicals with different reactivities. Once formed, radicals can interact with other radicals or molecules in numerous ways. Reaction speed is determined by concentration of the radical. Antioxidants, on contrary, are of interest to biomedical researchers and practitioners because they shield the body against reactive oxygen species damage. The antioxidants capacity to contribute electrons that can neutralise the radical generation helps to reduce and prevent damage from free radical reactions (Ganguly et al., 2021).

Increased blood glucose level caused diabetes mellitus which leads to complications such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases. Deficient insulin production in the body is a hallmark of type I diabetes, which is caused by the loss of pancreatic islet beta cells. To control blood glucose levels and, consequently, DM1, these patients use insulin on a daily basis. The DM type II is a non - insulin dependent and seen majority among the people around the world, results from the body’s ineffective hypoglycemic agent are used for controlling type II diabetes (Spellman.,

2010). Secondary metabolites include phenolic acids, glycosides, saponins, tannins, and others are responsible for the antidiabetic effects of medicinal plants. Plant growth circumstances, techniques for collecting, drying and storing plants, as well as techniques for extracting and purifying active chemicals, can all have an impact on antidiabetic effectiveness (Sukhikh et al., 2023).

Test Organisms for Anti-Bacterial Activity

Current antibacterial investigation focused on gram-positive bacteria(GPB) including *Bacillus subtilis* and *Staphylococcus aureus*, as well as gram-negative bacteria(GNB) including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescens*. Indian Punjab's Microbial Type Culture Collection provided bacterial strains mentioned above. Bacterial strains have been grown in nutrient broth then stored at 40°C until their antibacterial properties were assessed. Paper disc diffusion method has been employed for testing antibacterial properties.

Antimicrobial Susceptibility Testing

Pathogenic bacteria were spread out on nutrient agar plates in order to evaluate antimicrobial susceptibility testing. The root extracts from *Toddalia asiatica* and *Evolvulusalsinoides* were put on discs and incubated for 24hours at 30±2. Incubating for 24hours in a distinct zone of bacterial inhibition was observed around sample, and size of this zone of antibacterial activity was evaluated. Degree of inhibition is correlated with diameter of inhibition zone, that is measured in millimeters(ml), utilizing transparent ruler. Antibacterial activity has been compared utilizing ampicillin, an established antibiotic. At least three repetitions of each experiment had been performed.

ANTIOXIDANT ACTIVITY

DPPH Free Radical Scavenging Assay

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay has been employed for examining methanolic extracts from roots of *T. asiatica* and *E. alsinoides*for their capacity to scavenge free radicals. Different quantities of methanolic extracts (500,250,100,50, and 10µg/ml) from the roots of *T. asiatica* and *E. alsinoides* were mixed with a 0.1Mm solution of DPPH in methanol. Following permitting this mixture to react for thirty minutes at room temperature, absorbance at 517nm has been determined employing UV-visible spectrophotometer. Kapewangolo et al. (2015) stated, each experiment comprised an appropriate blank, and there were parallel positive controls that did not include the extract. As a standard, ascorbic acid at different levels was used.

Applying following formula, extract's scavenging activity has been calculated as proportion of DPPH radicals scavenged:

$$\% \text{ of scavenging activity} = [A\text{-}B / A] \times 100$$

A – absorbance of control, B- absorbance of sample

Here, sample represents absorbance of sample solution and control represents absorbance of control solution, that contains all reagents except test sample. Results represented by mean±SE of three replicates. According to Chand et al. (2016), linear regression analysis has been employed for calculating the plant extracts' IC50 value, while percentage of scavenging activity displayed against concentration.

Hydrogen Peroxide Scavenging Assay

Jayaprakasha et al. (2004) described methodology with slightly modified for assessing hydrogen peroxide scavenging capabilities of the consecutive methanolic extracts from the roots of *T. asiatica* and *E. alsinoides*. In 1M phosphate buffer (pH 7.4), 43mM hydrogen peroxide solution (0.6mL) mixed with 500, 250, 100, 50, and 10µg/ml of plant extracts and ascorbic acid. Suresh et al. (2020) and Hoque et al. (2021) used phosphate buffer as the blank and measured the absorbance at 230 nm after 10 minutes.

Following formula has been employed for calculating the hydrogen peroxide scavenging activity:

$$\text{Hydrogen Peroxide activity} = [A\text{-}B/A] \times 100$$

A – absorbance of control, B- absorbance of sample

Nitric Oxide Radical Scavenging Activity

Plant extract had been evaluated for its capacity to scavenge nitric oxide radicals by inhibiting the generation of nitric oxide radicals from sodium nitroprusside in a phosphate buffer saline solution using the Griess reagent, that is composed of 1% sulfanilamide, 2% orthophosphoric acid, and 0.1% naphthyl

ethylenediamine dihydrochloride. For 5-hours, experiment's sodium nitroprusside (0.6ml, 5mM) solution incubated at 25±20C with various amounts of extracts or ascorbic acid (1ml, 10-200 µg/ml). Senguttuvan et al. (2014) state that following incubation, 2 ml of reaction mixture was combined with an equivalent volume of Griess reagent, and absorbance of purple dye chromophore measured at 540nm employing UV-Vis spectrophotometer.

Nitric oxide radical scavenging ability quantified applying formula:

Nitric oxide activity (%)=[A-B/A] × 100

A – absorbance of control, B- absorbance of sample

This formula calculates percentage of inhibition based on the difference in absorbance between the control (absence of extract or Ascorbic acid) and the sample, providing insight into the antioxidant capacity of the leaf extract.

Anti-inflammatory Activity (Inhibition of Albumin Denaturation)

With a few minor modifications, the methodology described by Sakat et al. (2010) was used to evaluate the leaf extract's anti-inflammatory potential by inhibiting albumin denaturation. Test sample, designated K, had been mixed with 1% bovine serum albumin (500µL) at different concentrations (500, 250,100,50, and 10µg/ml) in this investigation. The final blend was permitted to be represented by the letter K. After ten minutes of standing at room temperature, the resultant mixture was heated for twenty minutes at 51^0C. Absorbance at 660nm then measured after solution had cooled to room temperature. In parallel trials, acetylsalicylic acid employed as positive control. Experimental procedure conducted in triplicate, and percentage inhibition of protein denaturation has been calculated. Assessments were made by comparing the results with the positive control (acetylsalicylic acid). This approach provides insights into potential leaf extract anti-inflammatory activity by evaluating it could inhibit albumin denaturation, a key marker in inflammation studies.

Protection (%)=100–[Optical density of Test sample/Optical density of control]×100

Antidiabetic Study

In Vitro Alpha Amylase Inhibitory Assay

Nitro amino salicylic acid, a brown-colored substance, is created when dinitro salicylic acid reacts with the reducing sugars produced by α amylase. Range of sample concentrations, from 6.25-100µg/mL, incubated for 10min at 25^0C. A 1000µl solution of 25m phosphate buffer pH 6.9, including 25µl of porcine α amylase at concentration of 0.5mg/ml, was prepared employing stock concentration of 10mg/mL. A reaction-stopping 50µl of 96Mm 3,5 dinitrosalicylic acid color reagent utilized. Incubating in boiling water bath lasted 5min, then microplate was left to cool to room temperature. A microplate reader was used to identify absorbance at 540 nm. (Wickramaratne et al, 2016)

% Inhibition = Control – test / Control × 100

In Vitro Alpha Glucosidase Inhibitory Assay

The amount of reducing sugar that results from the alpha glucosidase enzyme hydrolyzing sucrose was used to quantify alpha glucosidase activity. Sample effects measured employing slightly modified version of Matsui et al. (2004) approach. At a stock concentration of 10mg/mL, different sample volumes ranging from 6.25-100µg were acquired before the reaction started employing sucrose (37mM) as substrate in a final reaction mixture of 1mL of 0.1M phosphate buffer (pH 7.2). Samples then incubated for 5min. The process terminated by immersing reaction mixture in a boiling water bath for two minutes following a half-hour incubation period at 370°C for Phosphate buffer and an enzyme tube were maintained as a control. A microplate reader had been utilized to measure absorbance at 510nm after tubes were filled with 250µL of glucose reagent and incubated for 10min. Acarbose was used as the standard.

% Inhibition = Control – test / Control × 100

Statistical Analysis

The mean ± standard deviation is used to express the results. Using SPSS software, the data statistically analyzed using one-way ANOVA and Newman-Keuls Multiple Comparison to see if there were any significant differences between the methanol extracts of two plants and standards, with a criteria of P-values<0.05.

Results and Discussion

Anti-bacterial activity assessment of methanolic extracts of *T. asiatica* and *E. alsinoides* root in non-MDR strains suggest that both extracts exhibited remarkable anti-microbial activity against all the strains. Highest anti-bacterial activity was noticed by *E. alsinoides*plant extract against *S. aureus* (zone of inhibition is 14.0±0.1mm) and least against *Escherichia coli* (zone of inhibition is 9.0±0.1mm). When compared with standard control, percentage activity of *E. alsinoides*against *S. aureus* is 43% and *K. pneumonia* is 39%. On the other hand, highest anti-bacterial activity was witnessed by *T. asiatica* root extract against *Staphylococcus aureus* (zone of inhibition is 12.1 ± 0.1 mm) least against *Pseudomonas fluorescens* (zone of inhibition is 1.1 ± 0.2 mm). Compared with standard drug the percentage activity of *T. asiatica* root extract against *S. aureus* is 37% and *E. coli* is 36% (Table 1).

Table 1: Anti-bacterial Activity of Methanolic extracts of *Evolvulusalsinoides* and *Toddalia asiatica* root

Test Organisms	**Zone of Inhibition (mm)**		
	Control (Ampicillin)	***Evolvulusalsinoides*Extract**	***Toddalia asiatica* root Extract**
Klebsiella pneumonia	13.2 ± 0.2	3.2 ± 0.1	33.2 ± 0.1
Escherichia coli	12.6 ± 0.1	11.2 ± 0.3	30.2 ± 0.3
Pseudomonas fluorescens	13.2 ± 0.3	1.1 ± 0.2	35.1 ± 0.4
Bacillus subtilis	10.2 ± 0.1	2.4 ± 0.2	31.2 ± 0.3
Staphylococcus aureus	14.0 ± 0.2	12.1 ± 0.2	32.0 ± 0.5

Antioxidant Activities

Table 2: Effect of Methanol Extract of *Evolvulusalsinoides*on Anti-oxidant Activity

S.No	***Evolvulusalsinoides* extract Conc. µg/ml and IC_{50} value**	**Percentage of Inhibition (%)**		
		DPPH Assay	**H_2O_2 Assay**	**NO Assay**
1.	Ascorbic acid	99.12	99.23	91.46
2.	500	77.20	73.49	57.57
3.	250	71.71	70.42	49.97
4.	100	61.12	68.67	43.41
5.	50	46.52	67.24	38.15
6.	10	34.28	20.05	29.28
7.	IC_{50} (µg/ml)	79.24	28.62	47.35

Table 3: Effect of Methanol Extract of *Toddalia asiatica* root on Anti-oxidant Activity

S.No	*Evolvulusalsinoides* extract Conc. µg/ml and IC_{50} value	Percentage of Inhibition (%)		
		DPPH Assay	H_2O_2 Assay	NO Assay
1.	Ascorbic acid	99.31	98.43	94.15
2.	500	79.10	77.40	78.29
3.	250	74.21	69.22	75.76
4.	100	62.46	62.17	60.14
5.	50	48.37	57.44	46.45
6.	10	37.28	30.15	29.32
7.	IC_{50} (µg/ml)	80.14	38.12	51.07

DPPH Assay

The DPPH assay is widely used for antioxidant activity screening due to its rapid detection capability of active ingredients at low concentrations. The decrease in absorbance of the DPPH radical, resulting hydrogen donation, led to a visible color change across various concentrations (500,250,100,50 and 10µg/ml), with a reduction from 500g/ml to 77.20% for *E. alsinoides*plant extract (Table 2)and that for *T. asiatica* root extract is 79.10%. (Table 3)

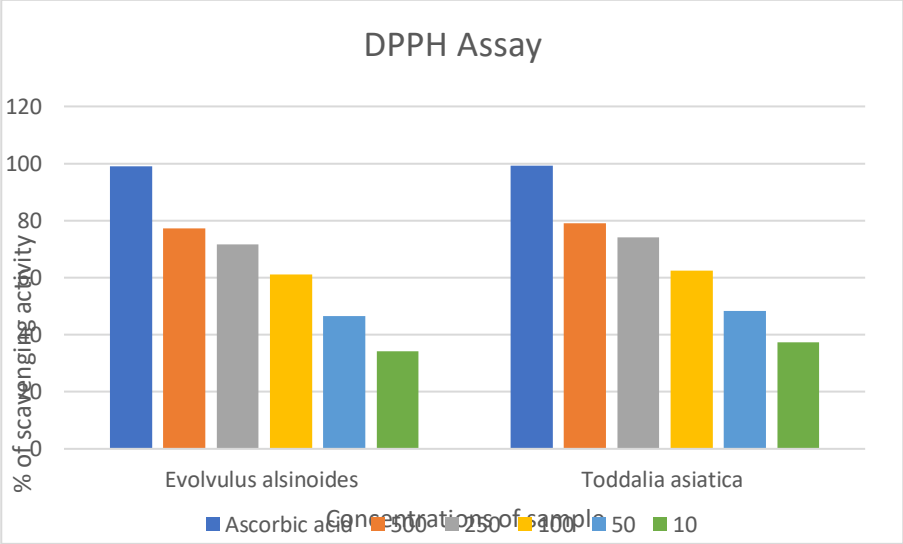

Figure 1: DPPH Scavenging Activties of methanolic extracts of *E. alsinoides* and *T. asiatica* root

The result showed that the methanol extract of *E. alsinoides*plant had Hydrogen peroxide scavenging activity (Figure 2). *E. alsinoides*significantly inhibited Hydrogen peroxide in various concentrations (500,250,100,50 and 10µg/ml) and in high concentration 500 µg/ml had 73.49% and an IC_{50} value of 28.62 100 µg/ml. Like that, the methanol extract of *T. asiatica* root had Hydrogen peroxide scavenging activity. *T. asiatica* root also significantly inhibited Hydrogen peroxide in varying concentrations (500,250,100,50 and 10µg/ml) and in high concentration 500 µg/ml had 77.40% and an IC_{50} value of 38.12µg/ml. Whereas the results indicate that the methanolic extracts of *E. alsinoides* and *T. asiatica* root might contain compounds capable of inhibiting Hydrogen peroxide, there is many experimental proofs for the use of the drugs in the indigenous system of treatment of various diseases.

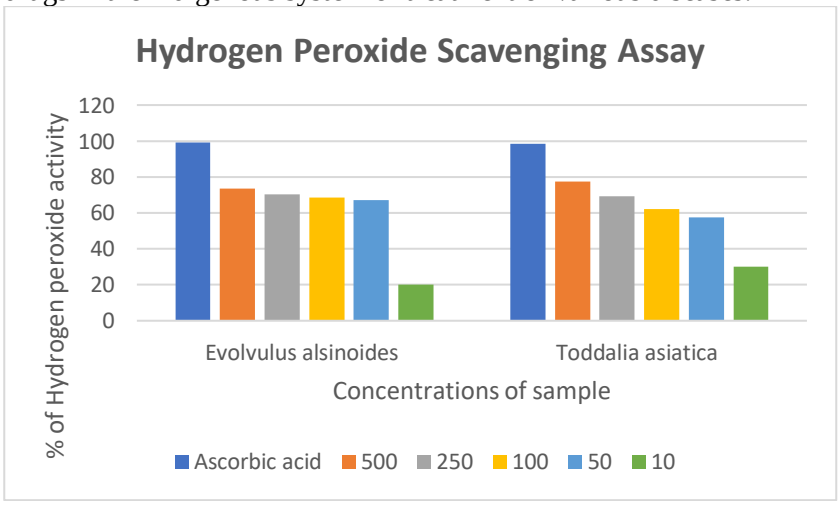

Figure 2: Hydrogen Peroxide scavenging activities of methanolic extracts of *E. alsinoides* and *T. asiatica* root

The impact of methanol extracts of *E. alsinoides* and *T. asiatica* root on nitric oxide scavenging activity showed in Figure 3. The study revealed significant inhibition of nitric oxide at various concentrations of *E. alsinoides* (500,250,100,50 and 10µg/ml), with the highest inhibition observed at 500 µg/ml (57.57%) and an IC50 value of 47.35 µg/ml and that *T. asiatica* root also showed significant inhibition of nitric oxide at various concentrations (500,250,100,50 and 10µg/ml), with the highest inhibition observed at 500 µg/ml (78.29%) and an IC50 value of 51.09µg/ml. These findings suggest the presence of a compound in the methanolic extracts of *E. alsinoides* and *T. asiatica* root have the potential to inhibit nitric oxide, providing scientific support for the traditional use of this plant in indigenous systems for treating various diseases.

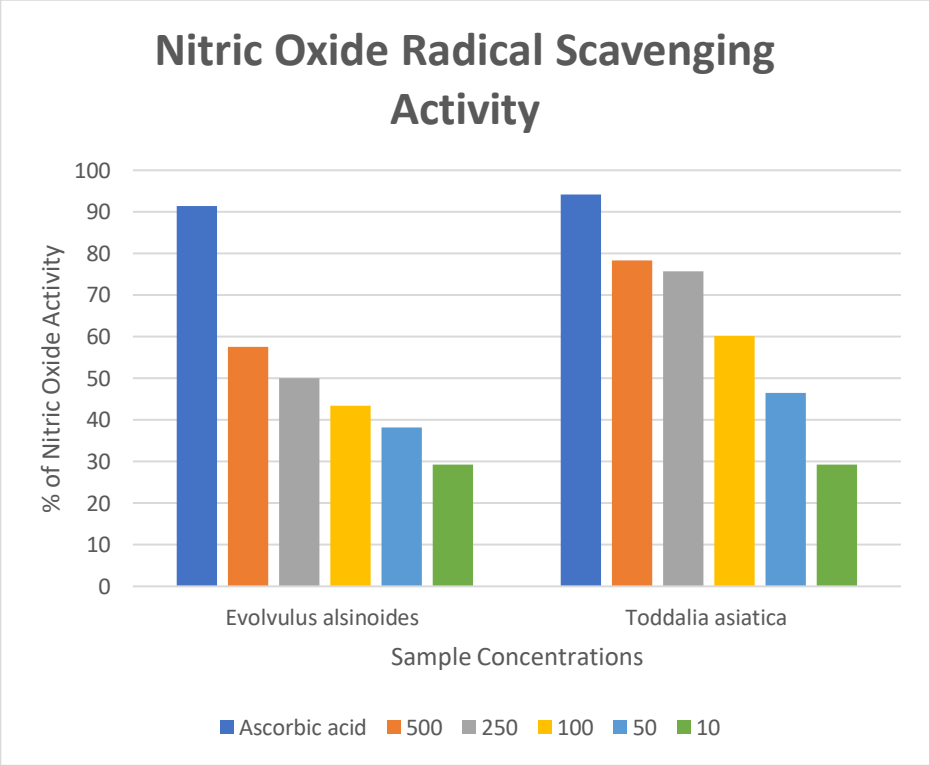

Figure 3: Nitric oxide radical scavenging activities of methanolic extracts of *E. alsinoides* and *T. asiatica* root

Anti-inflammatory Activity

Current investigation, examined impact of *E. alsinoides* and *T. asiatica* root methanol extract under various external stress conditions, including exposure to acid, base, heat and organic solvents. The study focused on the ability of *E. alsinoides* and *T. asiatica* root to mitigate protein denaturation, a process that can lead to inflammation as proteins lose their structure. Here, Acetylsalicylic acid serving as the standard reference. The results of inhibition activities of *Evolvulusalsinoides*of 61.70%, 72.30%, 84.21%, 87.96% and 95.33% across five concentrations (10,50,100,250,and 500µg/ml) respectively. Similarly, *T. asiatica* root showed the inhibition activities of 49.43%, 61.41%, 73.15%, 79.16% and 84.30% across five concentrations (10,50,100,250,and 500µg/ml) respectively. (Figure 4)

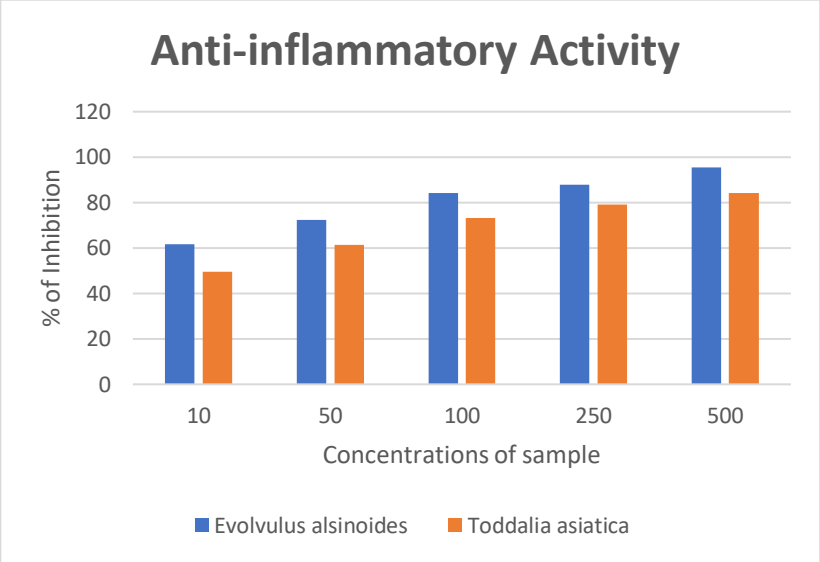

Figure 4: Anti-inflammatory activity of methanolic extracts of *E. alsinoides* and *T. asiatica* root

***In vitro* Antidiabetic Studies**

The inhibitory effect of *Evolvulusalsinoides* and *Toddalia asiatica* root extracts on *in vitro* α-amylase as well asα-glucosidase inhibition tests were performed. The half maximal inhibitory concentration (IC50) values of alpha amylase are 54.32 µg/mL for *Evolvulusalsinoides* methanolic extract and for *Toddalia asiatica* root extract was 75.61 µg/mL, while the standard acarbose with IC50 of 76.17. In case of alpha amylase inhibitory effect, the half maximal concentration (IC50) value for *Evolvulusalsinoides* methanolic extract is 59.92 against the standard acarbose with 28.98µg/mL and that of 83.49 µg/mL for *Toddalia asiatica* root extract. The results were illustrated in (Table 4, 5).

Table 4: α-Amylase Inhibitory Activity and IC50 (µg/mL) Values of *Evolvulusalsinoides* and *Toddalia asiatica* root Extracts

Sample Concentration(µg/mL)	***Evolvulusalsinoides*Extract**	***Toddalia asiatica* root Extract**
6.25	10.78±0.46	8.92±0.67
12.5	26.60±0.32	18.74±0.45
25	37.10±0.87	36.63±0.48
50	49.68±0.23	46.75±0.36
100	92.34±0.98	55.40±0.23
IC50	54.32	75.61

Table 5: α-Glucosidase Activity and IC50 (µg/mL) Values of *Evolvulusalsinoides* and *Toddalia asiatica* root Extracts

Sample Concentration (µg/mL)	***Evolvulusalsinoides*Extract**	***Toddalia asiatica* root Extract**
6.25	11.25±0.56	7.78±0.34
12.5	29.86±0.43	24.22±0.67
25	39.26±0.34	30.58±0.23
50	46.58±0.89	41.91±0.21
100	90.82±0.43	53.57±0.67
IC50	59.92	82.49

CONCLUSION

Current research reveals *in vitro* antioxidant properties of methanol extract of *E. alsinoides* and *T.asiatica* root, as evaluated by the DPPH assay, hydrogen peroxide and nitric oxide radical scavenging assay, as well as the in vitro anti-inflammatory, anti-bacterial and anti-diabetic activity. The findings suggest that these two plants possess promising potential for application in herbal medicinal therapies and demonstrates notable pharmacological and pharmaceutical activities.

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