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RESEARCH ARTICLE

Determination of Total Phenolic and Flavonoid Content, Antidiabetic, and Antioxidant Activities of Leaves and Seeds Extracts of *Eucalyptus* robusta Sm. and *Ageratina adenophora* Spreng

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Abstract

The present study aimed at the determination of total phenolic and flavonoid content and biological activities of *Eucalyptus robusta* (Sm.) and *Ageratina adenophora* (Spreng.) growing in the Kathmandu Nepal. People have been using these medicinal plants for many years against infectious diseases and to control diabetes. The extraction of plant secondary metabolites was done by the cold percolation method. The total phenolic (TPC) and flavonoid content (TFC) were quantified by the Folin-Ciocalteu phenol reagent and aluminium chloride colorimetric method. The α-amylase enzyme inhibition activity was performed to evaluate the antidiabetic property of plant extracts. The antioxidant potential of plant extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The methanol extract of *Ageratina Adenophora* Spreng leaves are found rich in plant secondary metabolites of yield percentage 36.83% whereas *Eucalyptus robusta* Sm. leaves and seeds have the yield percentage of 30.75% and 27.12% respectively. The extract of *E. robusta* leaves showed the highest phenolic content (200.89±6.67 mg gallic acid equivalent/g of dry extract) and *A. Adenophora* Spreng. leaves extract showed the flavonoid content (0.49±0.02 mg quercetin equivalent/g of dry extract) among the three samples studied, respectively. The extract of *E.robusta* Sm. seeds showed the strongest DPPH radical scavenging activity with a half-maximal inhibitory concentration IC_{50} of 110.8±1.73 µg/mL. The extract of *A. Adenophora* Spreng. was found potent towards α-amylase enzyme inhibition activity of IC_{50} 16.05±0.24 µg/mL whereas *E. robusta* Sm. leaves and seeds extracts showed IC_{50} 21.93±1.24 mg/mL and 21.82±0.09 mg/mL respectively. This study showed the leaves and seeds extracts of these two medicinal plants are found rich in phenolic and flavonoid compounds. These medicinal plants could be used to isolate the natural antioxidant and antidiabetic compounds that may be potential drug candidates in the future drug discovery process. This study support to provide the scientific validation for using these medicinal plants against the diabetes.

Keywords: *Eucalyptus robusta* Sm., *Ageratina Adenophora* Spreng. DPPH, antioxidant, antidiabetic

1. Introduction

Nepal is rich in natural resources and biodiversity where the medicinal plants growing in different altitudes are the second most bioresources after hydro resources. People living in different communities of Nepal have been using such medicinal plants since many years for the treatment of simple to life-threatening diseases. The plants play a significant role in human welfare in various ways like providing food, shelter, medicine, and completing the ecosystem in nature. From the prehistoric period, human beings have been using plants and their products for the healing and control of various diseases. The World Health Organization (WHO) has reported more than 80% of people in the world are depending on traditional therapy to cure different diseases [1]. Nowadays, medicinal plants are used not only as a traditional medicine but also have a great demand to

investigate and explore the possibilities of finding active pharmaceutical compounds that could be the drug candidate in the future drug discovery process. Among the different plants growing, aromatic plants are the rich sources of essential oils, terpenoids, generally monoterpenes and sesquiterpenes, as well as sometimes diterpenes, derivatives of aromatic compounds and volatile organic compounds, due to which are frequently used as medicine in traditional medicinal practices [2].

Medicinal plants have been used as a source of natural antioxidants to scavenge the free radicals generated in the body of human beings due to oxidative stress. These free radicals start the chain reactions which ultimately lead to the development of cancer, heart diseases,−aging, cataracts, and impairment of the immune system [3]. Polyphenols and flavonoids are secondary metabolites that show a significant role as an antioxidant generally involved in the body of human beings for defense mechanisms against ultraviolet radiation or aggregation by pathogens. The position and number of hydroxyl groups on the phenolic compounds are possibly related to their relative toxicity towards micro-organisms since increased hydroxylation results in increased toxicity. The antioxidant activity of the phenolic compound is mainly due to their redox property which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators [4].

The flavonoid-rich natural product inhibits the growth of microorganism and also have protective effects against many infectious and degenerative diseases such as cardiovascular and cancers. Flavonoid compounds isolated from medicinal plants are found active against cardiovascular diseases and act as antioxidant, anti-inflammatory, antimicrobial, antiallergic, and cytotoxic antitumors [5]. Diabetes mellitus has emerged as a major health problem in both developed and developing countries. It is a chronic endocrine metabolic disorder characterized by altered carbohydrates, lipids, proteins, electrolytes, and water metabolism. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas does not produce enough insulin or cells do not respond to the produced insulin [6]. The medicinal plants are found the potent natural α-amylase and α-glucosidase inhibitors which can be used as effective therapeutic agents for treating postprandial hyperglycemia with minimal side effects [7].

In this study, the leaves of *Ageratina Adenophora* Spreng. and the leaves and seeds of *Eucalyptus robusta* Sm.were collected from the Kathmandu district of Nepal to investigate the antihyperglycemic, antibacterial, and antioxidant activity along with the quantification of total phenolic and flavonoid content. Plant *A. Adenophora* Spreng. is commonly known as Cat weed is many stemmed, perennial herbaceous shrubs. The plant is used in India as an antiseptic, blood coagulant and to treat jaundice and ulcers. The juice of the plant is used in cuts and wounds. The juice of the root is used to treat the fever and the paste of the leaves is used to cure burnt and is also applied to treat eye insomnia.[8,9].

Eucalyptus robusta Sm. has been widely distributed in many countries around the world. The essential oils extracted from the leaves of the plant has widespread biological activities including antimicrobial,

Ageratina adenophora Spreng

Eucalyptus robusta Sm

Seeds of *Eucalyptus robusta* Sm.

Dry leaves of *eucalyptus robusta* Sm.

Figure 1: *Photographs of plant samples collected for the study.*

antiseptic, antioxidant, chemotherapeutic, respiratory and gastrointestinal disorder, wound healing and insecticidal. Recent studies have reported that the extract of *E. robusta* Sm. can be used for anticancer activity against some cancer cells such as colon, lungs, prostate, ovary, cervix, liver, and neuroblastoma [10,11]. To the best of our knowledge, this is the first attempt that we performed some selected biological activities, estimation of total phenolic and flavonoid content of the leaves and seeds of *E. robusta* Sm. and *A. Adenophora* Spreng. growing in the Kathmandu district of Nepal.

2.1 Materials and Methods

2.1 Chemicals

Most of the chemicals used in this research were of analytical grade of Sigma-Aldrich, New Delhi, India. Some important chemicals and reagents used in this study were methanol, acetone, hexane, dimethyl sulphoxide (DMSO), Folin-Ciocalteu phenol reagent (FCR), gallic acid, quercetin, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), $NaNO_2$, $AlCl_3$, and NaOH were of Sigma Aldrich purchased from the local city Kathmandu, Nepal. The reagents used during phytochemical analysis were prepared by using solvents of analytical grade and double-distilled water. The working solution of α-amylase and acarbose of (Sigma Aldrich) in desired concentrations was prepared by successive dilutions of the corresponding stock solutions throughout the experiment.

2.2 Equipment

The major equipment and glassware used in this research were a grinding mill, pipettes, micropipettes, weighing balance (Metler Toledo, ME204), hot air oven (Yamato Scientific DF412), and rotatory evaporator (Buchi R200), water bath (Buchi R200), 96 well plates reader, spectrophotometer.

2.3 Collection, identification, and preparation of samples

The leaves of *A. Adenophora* Spreng. (mature and immature) and the leaves and seeds of *E. robusta* Sm. were harvested in August 2018 from Kirtipur, Kathmandu Nepal, and the plants were authenticated at National Herbarium and Plant Laboratories, Godavari, Lalitpur, Nepal. The voucher specimen was deposited in the same department. The plants were identified as *Ageratina Adenophora* (Spreng.) R. King & H. Robinson belongs to the family Asteraceae with voucher code no. S. Regmi S-01 (KATH), and *Eucalyptus robusta* (Sm.) belong to the family Myrtaceae with voucher code no. S. Regmi S-01 (KATH), respectively**.** The plant materials were washed in tap water to remove the contaminants. Then the leaves and seeds were shade-dried to constant weight. The shade-dried leaves and seeds were ground into powder form in an electric grinder then packed in a sealed container and stored at 4 °C until required.

The phytochemicals present in the powdered leaves and seeds were extracted by the cold percolation method using methanol as a solvent. The powdered leaves and seeds (100 g) were kept separately in clean and dry conical flasks. Methanol (300 mL) was added to each flask and kept for 72 hours with frequent shaking. The mixtures were decanted and filtered with the help of Whatman No. 0.45 mm filter paper and thus obtained filtrates were concentrated in a rotatory evaporator under vacuum at 60 °C. The concentrated filtrates were kept in a beaker wrapped with aluminium foil containing small pores that allow facilitating the complete evaporation of the solvent. The percentage yield of the extract was calculated from the dry extracts.

2.4 Total phenolic content (TPC)

The total phenolic content in the extracts was quantified by Folin-Ciocalteu phenol colorimetric method based on the chemical reduction of reagent, a mixture of tungsten and molybdenum oxide. The total phenolic content was estimated by adopting the standard procedure described by Ainsworth et al. [12,13] with a slight modification. A calibration curve was constructed by using gallic acid as the standard. The different concentrations of samples were prepared by serial dilution and loaded in 96 well plates reader adding the FCR. The absorbance of the solutions was taken at 765 nm.

The total phenolic content was calculated using the following equation

$$C = \frac{cV}{m} \quad \ldots(1)$$

where, C= total phenolic compounds (mg GAE/g), c = concentration of gallic acid established from the calibration curve (mg/mL), V = volume of extract (mL), and m = weight of the plant extract (g). Data were recorded for each concentration, from which the linear correlation coefficient (R^2) was calculated.

$$y = mx + c \quad \ldots(2)$$

The concentration of the plant extract was calculated from the regression equation, Thus, with the calculated value of the concentration of the extract, the total phenolic content was calculated from equation (1). where, y = absorbance of the extract, m = slope from the calibration curve x = concentration of the extract, and c = intercept.

2.5 Total flavonoid content (TFC)

The total flavonoid content of the plant extracts was estimated by an aluminium chloride complex-forming assay. The flavonoid content was estimated using quercetin as standard [14]. The different concentrations of plant extracts and quercetin were prepared by serial dilution from the stock solution. The absorbance of the content on mixing with the reagent was measured at 415 nm after the incubation in dark for 30 minutes. The flavonoid content was calculated in mg QE/g by using the following equation.

$$C = \frac{cV}{m} \quad \ldots(3)$$

where, C= total flavonoid content (mg QE/g), c = concentration of quercetin established from the calibration curve (mg/mL), V = volume of extract (mL), and m = weight of the plant extract (g)

The data were recorded as absorbance for each concentration, from which the linear correlation coefficient (R^2) was calculated as,

$$y = mx + c \quad \ldots(4)$$

where, y = absorbance of the extract, m = slope from the calibration curve, x = concentration of the extract, and c = intercept

2.6 Antioxidant activity

The antioxidant activity of leaves and seeds extracts of plant samples were evaluated by the DPPH free radical scavenging assay described by Demirkiran et al. [15] with a slight modification. DPPH solution (0.1 mM) was prepared by dissolving 0.39 mg of DPPH in methanol and the final volume was made to 100 mL in a volumetric flask. The stock solution (100 µg/mL) of the extracts was prepared in dimethyl sulfoxide (DMSO) and the working solutions of concentrations 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.5 mg/mL were prepared by serial dilution. Then100 µLof the test solutions were mixed with 10 µL of DPPH solution in 96 well plate reader and the mixture of the solution was kept in dark for 30 minutes at room temperature. The control was prepared by mixing the methanol and DPPH. The absorbance was measured at 517nm. Ascorbic acid of the same concentrations was prepared as a standard and its absorbance was measured spectrophotometrically at 517nm. The radical scavenging activity was evaluated based on the percentage of DPPH radical scavenged as the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_s}{A_0} \times 100 \quad \ldots(5)$$

Where, A_0 = absorbance of the control (DPPH), A_s= absorbance of the test sample and DPPH.The results were reported as IC_{50} values as ascorbic acid equivalent for each extract of plant samples. The data were presented in triplicate, as mean values ± standard deviation (n=3).

2.7 α-Amylase enzyme inhibition

The antidiabetic activity of leaves and seeds extracts of plants was evaluated by the α-amylase inhibition assay described by Xiao et al. [24] with a slight modification. The undigested starch due to enzyme inhibition was detected through the blue starch iodine complex spectrophotometrically at 620 nm. The stock solution of the extracts was prepared by dissolving 500 mg in 1 mL DMSO. The working

solution of different concentrations was prepared by serial dilution of the stock solution.

The mixture composed of 20 µL 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 20 µL 4 units of PPA (Porcine pancreatic α-amylase) solution, and 20 µL plant extracts at a concentration from 1.56-50 mg/mL (w/v) were incubated at 37 °C for 10 minutes. Then 20 µL soluble starch 1% (w/v) was added to each reaction well and incubated at 37 °C for 15 minutes. 1 M HCl (20 µL) was added to terminate the reaction, followed by the addition of 100 µL of 5 mM iodine reagent. The color change was noted and the absorbance was read at 620 nm on a 96-well microplate reader. The control reaction was due to the enzyme only without the plant extract. A dark-blue colour indicates the presence of starch; yellow colour indicates the absence of starch while a brownish colour indicates partially degraded starch in the reaction mixture. The known PPA inhibitor, acarbose, was used as a positive control at a concentration range of 1.56-50 mg/mL. In the presence of inhibitors found in the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark blue colour complex whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase.

$$\% \text{ relative enzyme activity} = \frac{\text{Enzyme activity of test}}{\text{Enzyme activity of control}} \times 100 \quad \ldots(6)$$

% inhibition of α-amylase activity = (100 - % relative enzyme activity), Where, the enzyme activity of test = total starch – remaining starch, the enzyme activity of control = starch only – (starch + enzyme)

3. Results and Discussion

The percentage yield of the plant extracts is displayed in Table 1. The extracts of *A. adenophora* leaves showed the highest yield percentage (36.83%) and the *E. robusta* seeds have the lowest (27.12%), whereas the *E. robusta* leaves showed a moderate yield. The *A. adenophora* is a rich source of plant metabolites. The yield percentage of the extracts depends on the harvesting time, geographical distribution, solvent polarity, extraction procedure, temperature, and composition of the sample.

Table 1:*The percentage yield of plant samples.*

plant samples	weight of plants samples (g)	% yield
E. robusta leaf	25	30.75
E. robusta seed	22	27.12
A. Adenophora leaf	28	36.83

3.1 Total phenolic and flavonoid content (TPC and TFC)

The total phenolic content (TPC) in the extracts of leaf and seed of *A. Adenophora* and *E. robusta* is presented in Table 2.

The total phenolic content of the methanolic leaf and seed extract was calculated from the calibration curve (R^2 =0.973) by using gallic acid as standard. Among the extracts, the highest phenolic content was found in *E. robusta* seed (200.89±6.67 mg GAE/g dry weight) followed by *E. robusta* leaf (182.24±3.38 mg GAE/g) and *A. Adenophora* leaf (67.74±3.51 mg GAE/g).

The phenolic content in the *E. robusta* leaf was found higher (182.24±3.38 mg GAE/g) than the results reported by Vuonget et al. [16] of 125.8 ±1.42 mg GAE/g. The results of the present study show that the plant growing in Kathmandu, Nepal is found a rich source of phenolic compounds. The total flavonoid content in the methanolic extracts of *A. Adenophora* and *E. robusta* was calculated from the calibration curve (R^2 = 0.996) by using quercetin as the standard. The results of total flavonoid content are presented in Table 3.

Table 2:*Total phenolic content in leaf and seed extracts of plant samples (n=3).*

Plant samples	Absorbance			TPC (mg GAE/g)			Mg GAE/g ±SD
	A_1	A_2	A_3	C_1	C_2	C_3	
E. robusta leaf	0.339	0.330	0.342	183.24	178.36	184.86	182.24±3.38
E. robusta seed	0.382	0.358	0.375	206.48	93.51	202.70	200.89±6.67
A. Adenophora	0.132	0.119	0.125	71.35	64.32	67.56	67.74±3.51

Table 3: *Total flavonoid content in leaf and seed extracts of plant samples (n=3).*

Plant samples	Absorbance			TFC (mg QE/g)			mg QE/g ±SD
	A_1	A_2	A_3	C_1	C_2	C_3	
E. robusta leaves	0.171	0.183	0.199	0.331	0.354	0.385	0.35±0.02
E. robusta seeds	0.114	0.135	0.129	0.220	0.261	0.249	0.24±0.02
A.adenophora	0.268	0.253	0.243	0.518	0.489	0.470	0.49±0.02

Table 4: *The percentage of radical scavenging and the concentration of the plant extracts.*

Concentration (µg/mL)	% radical scavenging			
	***E. robusta* leaves**	***E. robusta* seeds**	***A. Adenophora***	**Ascorbic acid**
320	58.41±2.59	63.22±0.91	54.74±1.95	92.66±0.86
160	47.42±2.75	55.78±1.10	25.19±3.52	81.66±0.71
80	37.79±1.49	46.61±1.73	15.34±1.20	75.48±0.39
40	32.41±2.48	37.91±1.38	8.93±1.49	66.43±0.52
20	22.67±1.49	26.11±1.72	6.41±0.79	57.61±0.86
10	15.91±0.86	14.65±0.99	3.89±0.39	39.97±1.20

The leaf and seed extracts were found the poor sources of flavonoid content than phenolic content. The leaf extract of *A. adenophora* has a high flavonoid content (0.49±0.02 mg QE/g) as compared to the *E. robusta* leaf and seed extracts (0.35±0.02 mg QE/g and 0.24±0.02 mg QE/g) respectively. The flavonoid content in these samples was found less than the result reported by Tripathi et al. [25] of 32.25±1.15 mg QE/g in the methanolic leaf extract. It is due to the altitude variation and the environment of the plant growing. Similar results were reported by Kapali et al. [20] in the aerial parts of *A. adenophora* of 0.65±0.12 mg QE/g extract. The antioxidant activity of plant secondary metabolites such as flavonoids including flavones, flavonols, and condensed tannins depends on the presence of the free OH group and has *in vivo* and *in vitro* antioxidant activity [17,18].

3.2 Antioxidant activity

The medicinal plants rich in secondary metabolites such as phenolics, flavonoids, and carotenoids impart antioxidant activity due to their redox properties and chemical structure. The methanolic extracts of the leaf and seed of *E. robusta* and *A. Adenophora* showed strong antioxidant activity against the DPPH radical scavenging activity. The percentage of DPPH radical scavenging to the methanolic leaf extract of *E. robusta* and *A. Adenophora* was found lower than the seed extract of *E. robusta.* The comparison of the percentage of radical scavenging against the different concentrations of the extracts is displayed in Table 4. The radical scavenging of plant extracts is compared to the standard ascorbic acid as the positive control.

The linear regression of the percentage radical scavenging versus concentration was used for the calculation of the concentration of each plant extract required for 50% inhibition of DPPH activity (IC_{50}). The antioxidant potential has an inverse relation with the IC_{50} value, a lower value of IC_{50} indicates high antioxidant potential. The IC_{50} value of the plant extract along with the standard ascorbic acid is shown in Fig 2.

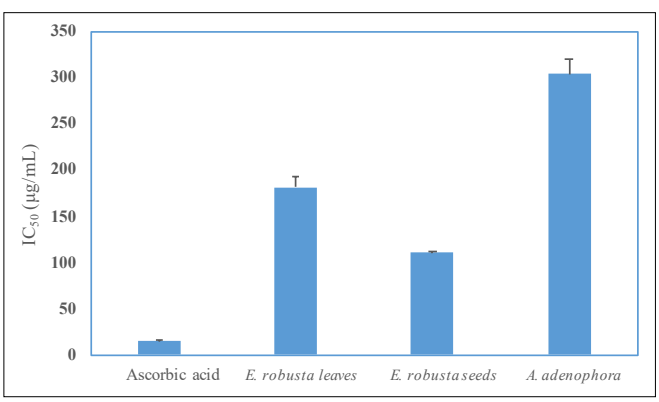

Figure 2: *DPPH radical scavenging shown by the plant extracts (IC_{50}).*

The inhibitory concentrations of plant extracts are found higher than that of the ascorbic acid used as standard. The present study showed methanolic extracts of *E. robusta* seed of IC_{50}110.8 ±1.73 µg/mL

have the strongest DPPH radical scavenging activity as their IC_{50} values were higher than the standard ascorbic acid of 15.64±0.76 µg/mL. Similarly, the IC_{50} values of methanolic extract of *E. robusta* leaf and *A. Adenophora* leaf were found to be 181.56±10.76 µg/mL and 303.6 ±15.95 µg/mL respectively showing moderate antioxidant activity. The leaf extract of *E. robusta* showed greater antioxidant activity as reported by Vitta et al. [19] of IC_{50} 423.14 ±73.27 µg/mL.

The antioxidant activity of *A. adenophora* reported by the previous researcher was found comparable to the results obtained in this study. Kapali et al. [20] have reported the antioxidant potential of *A. adenoophora* Spreng. aerial parts of IC_{50} 422.20 ±18.58 µg/mL. It is found that the leaf extract of *A. Adenophora* Spreng showed high antioxidant activity of IC_{50} 181.56±10.76 µg/mL as compared to the aerial parts of the plant as reported by Shukla et al., 2009. The formation of free radicals in the human body may cause cell damage and induces various physiological disorders. The superoxides and some other reactive oxygen species may damage cells and DNA, leading to various diseases [21]. The antioxidants stop oxidative damage at the cellular level of human beings by terminating the radical chain reactions. These plant extracts could be a promising source of natural antioxidants for isolating the potent chemical compound which could be the drug candidate for the future drug discovery process. The antioxidant potential of the plant extract is responsible for bioactivity due to its high phenolic and flavonoid content. Flavonoids are the potential radical scavenger of most oxidizing molecules mainly due to the singlet oxygen, and various other free radicals involved in several diseases [21].

3.3 Antidiabetic activity

The results of the concentrations of the plant extract to the percent inhibition of α-amylase experienced by the plant extracts are shown in Table 5. The α-amylase enzyme inhibition shown by the plant extracts was plotted against the concentrations of plant extracts from which the half-maximal inhibitory concentration IC_{50} was calculated graphically.

Table 5: *α-amylase enzyme inhibition and the concentrations of plant extracts.*

Concentration (mg/mL)	% inhibition of α-amylase		
	A. Adenophora leaves	*E. robusta* seeds	*E. robusta* leaves
50	76.76±1.60	62.21±0.09	62.79±2.62
25	67.29±0.12	60.86±0.15	59.50±13.41
12.5	39.45±0.24	37.34±0.13	35.25±5.03
6.25	26.95±0.16	22.02±0.13	26.07±6.18
3.125	10.65±0.20	10.81±0.14	14.85±3.21
1.5625	3.77±0.10	6.67±0.18	9.08±0.06

The inhibitory concentrations for the 50% inhibition of α-amylase by the plant extract are shown in Fig 3.

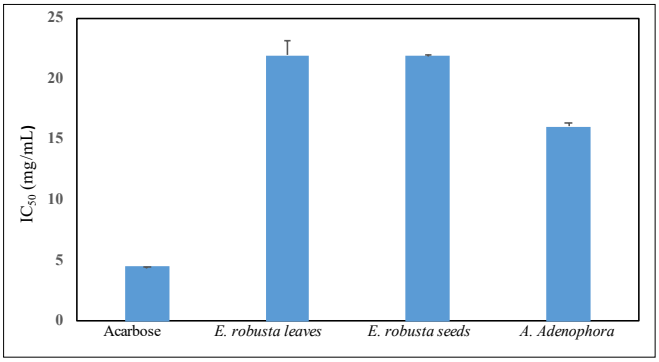

Figure 3: *α-Amylase inhibition activity (IC_{50}) of plant extracts and acarbose.*

The IC_{50} value of standard acarbose was found to be 4.46±0.08 mg/mL. Among these plants, methanolic extracts of *A.adenophora* showed potential *α*-amylase inhibition activity of IC_{50} value 16.05±0.24 mg/mL. Similarly, methanolic extracts of *E. robusta* leaf and seed have the IC_{50} of 21.93±1.24 mg/mL and 21.82±0.09 mg/mL respectively. The *A. adenophora* showed the highest percentage inhibition of α-amylase as compared to the leaf and seed extracts of *E. robusta*. It has been reported that the hepatic glucose output (HGO) inhibitory activity decreased in glucose output on oral administration of *A. adenophora* aerial parts (160 mg/kg, orally) in the Wistar rats [22]. The *α*-amylase enzyme inhibitors delay the breaking down of polysaccharides in the small intestine and diminish the postprandial blood glucose level in human beings suffering from diabetes. One of the strategies adopted to lower the levels of postprandial hyperglycemia involves the inhibition of carbohydrates and digestive enzymes such as *α*-amylase that lowers the absorption

of glucose to the blood through the gastrointestinal system [23].

The α-amylase enzyme inhibition activity was reported by Kapali et al. [20] on the aerial parts of the *A. Adenophora* Spreng. of 1.84±0.07 mg/mL. The results showed the aerial parts of the plant exhibited more α-amylase enzyme inhibition than the leaf extracts of 16.05±0.24 mg/mL. Kapali et al. 2021 have reported the α-amylase enzyme inhibition activity for methanol extracts of *Lantana camara* and *Cupressus sempervirens* of IC50 2.75±0.046 mg/mL and 4.86±0.20 mg/mL respectively. The present research showed *Lantana camara* and *Cupressus sempervirens* were found more potent than the *A. Adenophora* Spreng and *E. robusta* Sm to the α-amylase enzyme inhibition activity.

4. Conclusions

The results of the present research suggested that the leaf extract of *A. adenophora* has a high yield percentage of extracts showing rich sources of secondary metabolites. The *E. robusta* seed is rich in total phenolic content of 200.89±6.67 mg GAE/g dry weight, whereas the *E. robusta* and *A. adenophora* leaves showed a moderate amount of 182.24±3.38 mg GAE/g and 67.74±3.51 mg GAE/g respectively. The leaf extract of *E. robusta* is the rich source of the total flavonoid content of 0.49±0.02 mg QE/g whereas, the seed and leaf extracts of *E. robutsa* Sm. and *A. Adenophora* Spreng. showed a moderate amount of flavonoid content of 0.35±0.02 and 0.24±0.02 mg QE/g respectively. The *E. robusta* seeds are the potent sources of antioxidants of IC_{50} 110.8±1.73µg/mL showing high DPPH radical scavenging activity. The extracts of *E. robusta* Sm. and *A. Adenophora* Spreng. leaf showed the IC_{50} of 181.56±10.76 and 303.6±15.95µg/mL, the moderate antioxidant activity. The *A. Adenophora* Spreng. leaf extract showed the high α-amylase enzyme inhibition activity of IC_{50} 16.05±0.24 µg/mL as compared to the *E. robusta* Sm. leaf and seed have IC_{50} of 21.93±1.24 µg/mL and 21.82±0.09 µg/mL respectively. A further phytochemical analysis is required to isolate the chemical compounds from the leaf and seed extracts of *A. Adenophora* Spreng. and *E. robusta* Sm. which may show a broad spectrum of pharmacological activity.

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Data availability

The corresponding author has all the data for this study.

Conflicts of interests

All authors declare no conflicts of interest.

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