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Rashid Tajbin
Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

Zaman Sonia
Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

Howlader Saurav
Department of Food and Life Science, Pukyong National University, Busan, South Korea

Siraji Ismail Hossain
Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

Jony Md. Rasheduzzaman
Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

Corresponding Author:
Jony Md. Rasheduzzaman
Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh

In vitro antioxidant activity of *Antidesma bunius* leaf extracts

Rashid Tajbin, Zaman Sonia, Howlader Saurav, Siraji Ismail Hossain and Jony Md. Rasheduzzaman

Abstract
The aim of the present study was to evaluate the antioxidant potential of *Antidesma bunius* leaf extracts. Antioxidant activity was evaluated via DPPH scavenging assays. In addition, scavenging assays using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to evaluate antioxidant potential of *Antidesma bunius*. The leaf extracts of *Antidesma bunius* showed significant antioxidant effects in DPPH scavenging assays. Likewise, methanol (MeOH) extracts of *Antidesma bunius* exhibited potent inhibitory activities against DPPH with the corresponding IC_{50} values of 56.49±3.4 µg/mL, respectively. In addition, the highest DPPH scavenging activity was observed in the ethyl acetate (EASF) fraction with an IC_{50} value of 136.52 ± 0.03 µg/mL, followed by the CH_2Cl_2 fraction (DCMSF) with an IC_{50} value of 202.60 ± 0.09 µg/mL compared to ascorbic acid (IC_{50} = 43.04 ± 0.01 µg/mL). Our study establishes that *Antidesma bunius* extract might be useful as a potential antioxidant agent.

Keywords: *Antidesma bunius*, extraction, fractionation, 1,1-diphenyl-2-picrylhydrazyl (DPPH), antioxidant

Introduction
Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules *viz* nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc. [1-2]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders [3]. Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds *viz*. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione. Prior and Cao, reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals [4]. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psych neuropharmacology [5]. Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of *Antidesma bunius* leaf extracts.

Antidesma bunius is a genus of about 150 species distributed in Asia, Africa, Australia and Islands of pacific [6]. *Antidesma bunius* plant grows all over Bangladesh in many wet evergreen forest, dipterocarp forest, teak forest; at forest edges, on river bank, roadsides, in bamboo thickets; in semi-cultivated and cultivated areas. The whole plant is claimed to possess medicinal properties. The leaves are used as a traditional medicine for the treatment of skin disorder, syphilis and snakebites. It is also effectively used in indigestion, cough, stomachache, hepatoprotective and hepatotoxicity activities of *A. bunius* leaves [7-9]. The Fruits are healthy alcoholic juice drink and cooked with fish or other foods. A. bunius fruits contain anti-toxins which are traditionally used in the management of diabetes, hypertension, gastric intestinal problems, dysentery, indigestion, constipation, remedies for animals like sheep and goats [10-11]. Roots are used as antihelminthic and also recommended in cough and stomachache and indigestion. The bark of *A. bunius* Linn is traditionally used the bark for diabetic agent in Asia. Different extracts of *A. bunius* have been studied for possible biological activities in various animal models and reported to possess significant cytotoxic, anti-diabetic, anti-radical, anti-tumor, anti-inflammatory activities and so on. However, there have been no studies on *A. bunius* leaves responsible for antioxidant activities on different fractions. In this study, we aimed to determine the antioxidant activity of the *A. bunius*. Our data suggest that *A. bunius* may represent a source to prevention or treatment associated with oxidative damage-associated diseases.

Materials and Methods

Chemicals and Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). n-hexane, methanol, ethyl acetate, dichloromethane and chloroform were purchased from trusted sources. All other chemicals and solvents were purchased from trusted sources.

Plant Material

The plant sample of *Antidesma bunius* leaves were collected in August, 2016 from local area of Bangladesh. A voucher specimen was submitted to the National Herbarium, Mirpur, Dhaka, Bangladesh with accession number: DACB 43490. Plants were then washed properly to remove dirty materials and air-dried for several days. These were then ground with a hammer grinder for better grinding. The dried leaves were ground into a coarse powder. Then, the dried powder was preserved in an airtight container against the re-absorption of moisture, oxidation, excessive heat or humidity, growth of moulds and bacteria and infestation by insects and rodents.

Name of plant	Family	Plant part
Antidesma bunius	Euphorbiaceae	Leaves

Fig 1: Plant and leaves of *Antidesma bunius*

Preparation of *A. bunius* extracts

Powdered leaves materials having a weight of about 400 gm were taken in an amber colored reagent bottle and soaked in 1.75 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 60 °C temperature to afford crude extract.

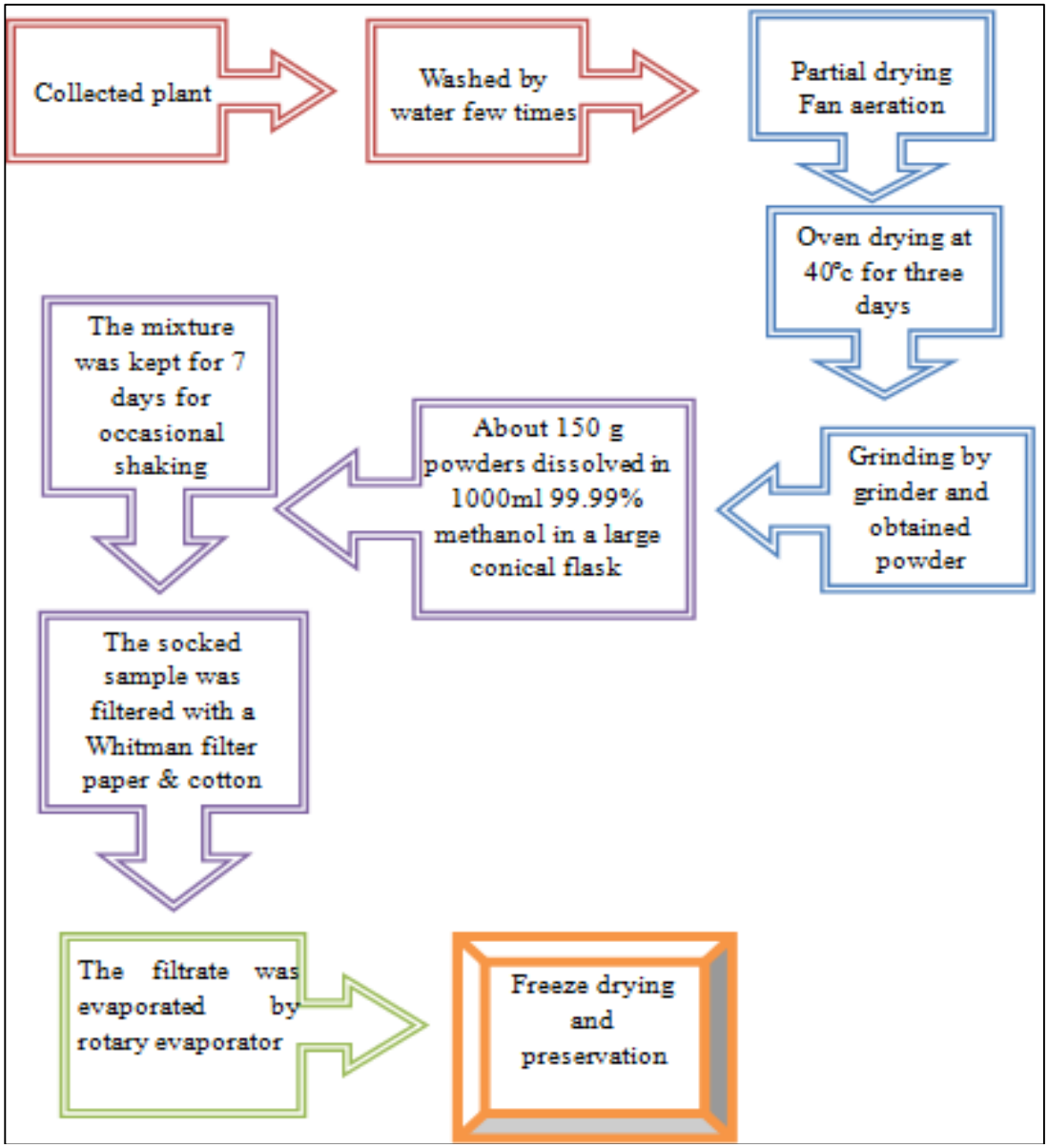

Fig 2: Schematic representation of preparation and extraction of crude extract of *Antidesma bunius*

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated using the method of Blois [12], with slight modification. Ascorbic acid was used as positive control. Calculated amount of ascorbic acid was dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration ranging from 500.0 to 0.977 µg /ml. Calculated amount of different extractives was measured and dissolved in methanol to get the mother solution (conc. 1000 µg/ml). Serial dilution of the mother solution gave different concentration ranging from 500.0 to 0.977 µg /ml which were kept in the marked flasks. 20 mg DPPH powder was weighed and dissolved in methanol to get a DPPH solution having a concentration 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light proof box. 2.0 ml of a methanol solution of the sample (extractives/ control) at different concentration (500 µg/ml to 0.977µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 520 nm using a VERSAmax microplate spectrophotometer (Molecular Devices). The antioxidant activity of both samples is expressed in terms of IC_{50} values (µg/mL, required to inhibit DPPH radical formation by 50%.

Inhibition of free radical DPPH in percent *(I%)* was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \; X \; 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Statistical analysis

Data are presented as the mean ± SEM of at least four independent experiments. Statistical comparisons between groups were performed using one-way ANOVA followed by Student's *t*-test. A *P* value less than or equal to 0.05 was considered statistically significant.

Results

Scavenging effect of DPPH from *A. bunius*

The methanol extract (ME) of properly dried and grinded leaves of *A.bunius* and its different fractions i.e. ethyl acetate (EASF), dichloromethane (DCMSF), N-hexane (NHSF) and aqueous (AQSF) soluble fractions were subjected to free radical scavenging activity by the method of Scavenging method with DPPH. The radical-scavenging activities of MeOH extracts from *A. bunius* are shown in Table 1. *A. bunius* showed potent activity in DPPH scavenging activity with an IC_{50} value of 56.49 ± 3.4 µg/mL as compared to the positive ascorbic acid control (IC_{50} = 43.04 ± 0.01 µg/mL). In this investigation, ME showed the highest free radical scavenging activity. The IC_{50} value was 56.49 ± 3.4 µg/ml. The other partionates like EASF, NHSF, DCMSF and AQSF exhibited good scavenging activity having IC_{50} values 136.52 ± 0.03 µg/ml, 202.6±0.09 µg/ml, 245.8 ± 0.08 µg/ml, and 305.0 ± 0.07 µg/ml, respectively. The leaves of *A. bunius* at 0.977-500 µg/ml concentration showed statistically significant antioxidant activity.

Table 1: Antioxidant activities of the methanolic extract from *A. bunius*[a]

Plant part	Sample code	Test sample	IC_{50} (µg/ml)
A. bunius (Leaves)	ME	Methanol extract	56.49 ± 3.4
	EASF	Ethyl acetate soluble fraction	136.52 ± 0.03
	DCMSF	Dichloromethane soluble fraction	202.6 ± 0.09
	NHSF	N-Hexane soluble fraction	245.8 ± 0.08
	AQSF	Aqueous soluble fraction	305.0 ± 0.07
ASA (Ascorbic acid) (standard)			43.04 ± 0.01

[a] The concentration that caused 50% inhibition (IC_{50}) is given as the mean ± SEM of triplicate experiments

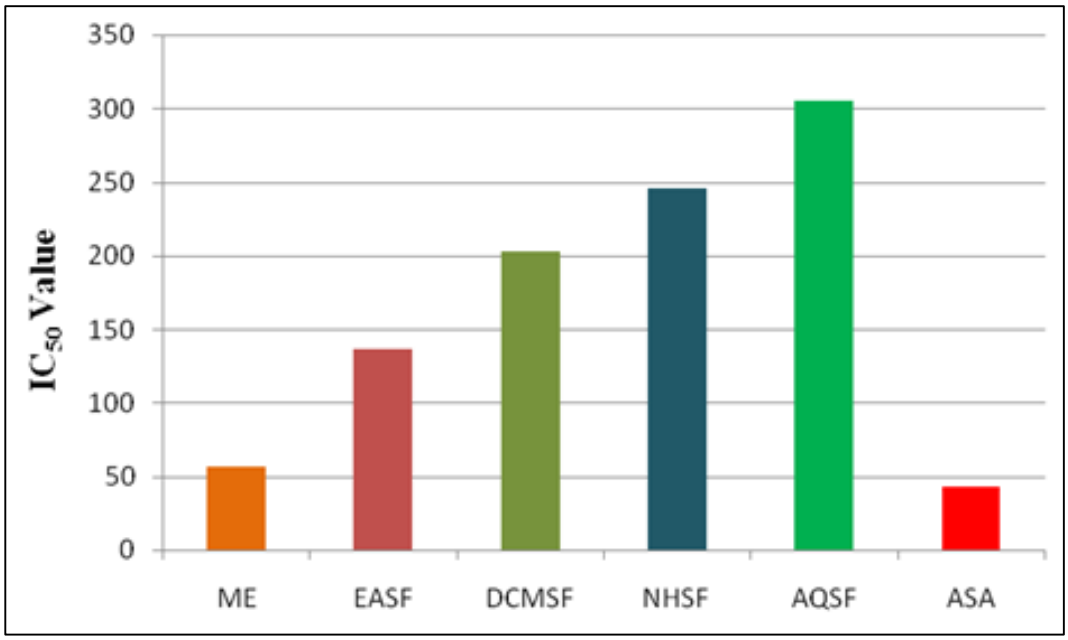

Fig 3: IC_{50} values of the standards and fractions of leaves of *A. bunius*

Table 2: IC_{50} value of ascorbic acid

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.005	98.46	43.04
	250	0.006	98.15	
	125	0.015	95.38	
	62.5	0.024	92.61	
	31.25	0.068	79.07	
	15.625	0.098	69.84	
	7.813	0.139	57.23	
	3.906	0.195	40.00	
	1.953	0.244	24.92	
	0.977	0.266	18.15	

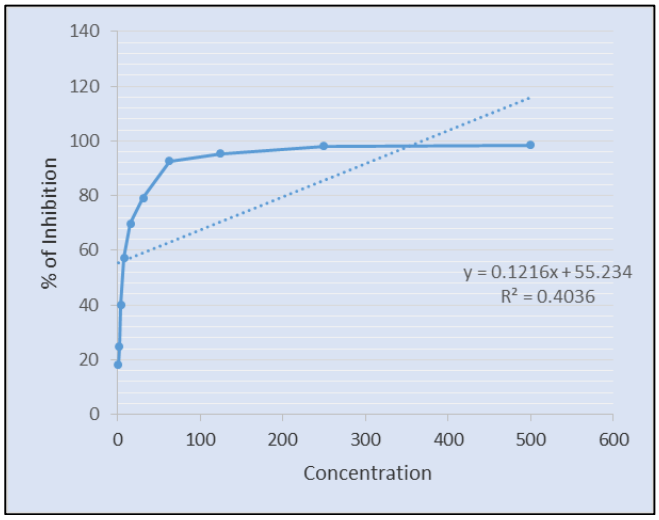

Fig 4: IC_{50} value of ascorbic acid

Table 3: IC_{50} value of methanol extract (ME) of leaves of *A.bunius*

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.131	59.69	56.49
	250	0.146	55.07	
	125	0.198	39.07	
	62.5	0.177	45.54	
	31.25	0.220	32.30	
	15.625	0.246	24.30	
	7.813	0.278	14.46	
	3.906	0.299	8.00	
	1.953	0.320	1.54	
	0.977	0.315	3.08	

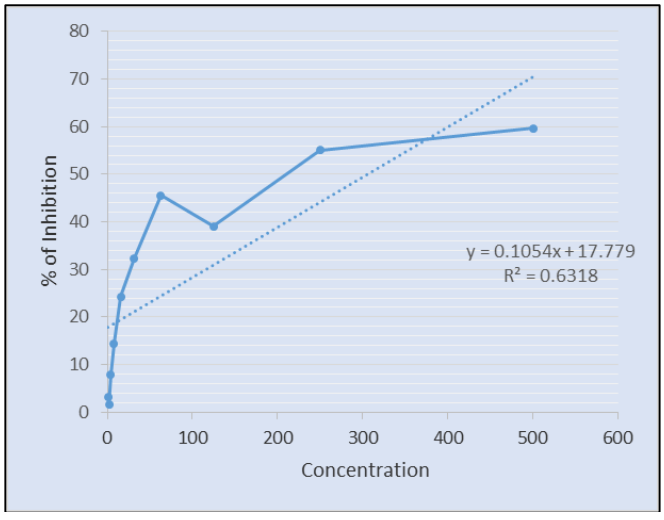

Fig 5: IC_{50} value of ME of leaves of *A. bunius*

Table 4: IC_{50} value of EASF of methanol extract of leaves of *A. bunius*

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.081	75.08	136.52
	250	0.106	67.39	
	125	0.128	60.62	
	62.5	0.126	61.23	
	31.25	0.154	52.62	
	15.625	0.178	45.23	
	7.813	0.189	41.85	
	3.906	0.220	32.31	
	1.953	0.258	20.62	
	0.977	0.298	8.31	

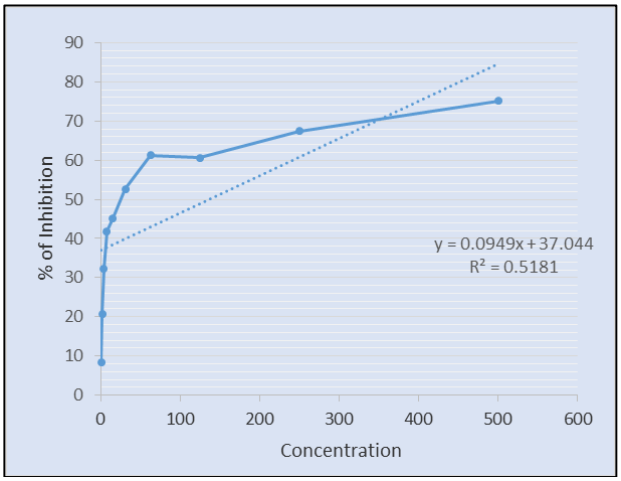

Fig 6: IC_{50} value of EASF of leaves of *A. bunius*

Table 5: IC_{50} value of DCMSF of methanol extract of leaves of *A. bunius*

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.062	80.92	202.6
	250	0.108	66.77	
	125	0.145	55.38	
	62.5	0.167	48.62	
	31.25	0.198	39.08	
	15.625	0.231	28.92	
	7.813	0.268	17.54	
	3.906	0.293	9.85	
	1.953	0.316	2.77	
	0.977	0.322	0.92	

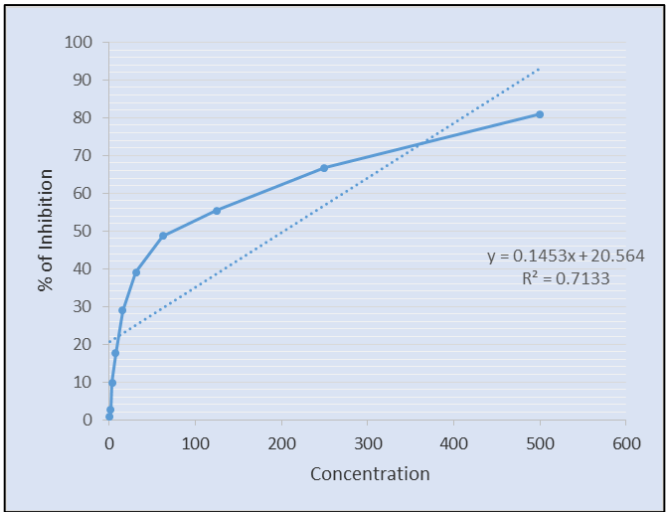

Fig 7: IC_{50} value of DCMSF of leaves of *A. bunius*

Table 6: IC_{50} value of NHSF of methanol extract of leaves of *A. bunius*

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.101	68.92	245.8
	250	0.146	55.08	
	125	0.167	48.62	
	62.5	0.154	52.62	
	31.25	0.188	42.15	
	15.625	0.221	32.00	
	7.813	0.246	24.31	
	3.906	0.291	10.46	
	1.953	0.314	3.38	
	0.977	0.321	1.23	

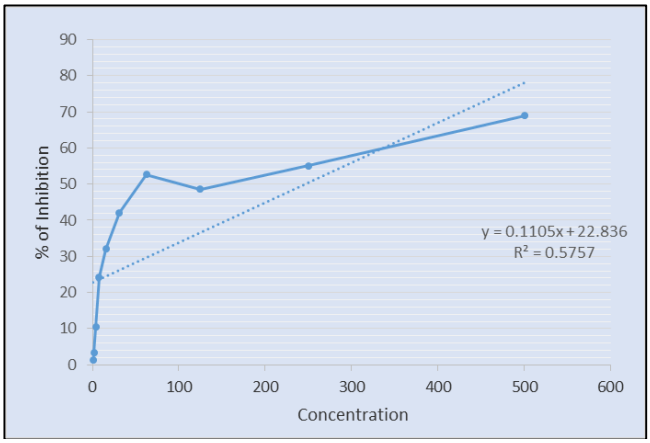

Fig 8: IC_{50} value of CSF of leaves of *A. bunius*

Table 7: IC_{50} value of AQSF of methanol extract of leaves of *A. bunius*

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.325	500	0.054	83.38	305.00
	250	0.068	79.08	
	125	0.079	75.69	
	62.5	0.094	71.08	
	31.25	0.120	63.08	
	15.625	0.158	51.38	
	7.813	0.176	45.85	
	3.906	0.201	38.15	
	1.953	0.224	31.08	
	0.977	0.269	17.23	

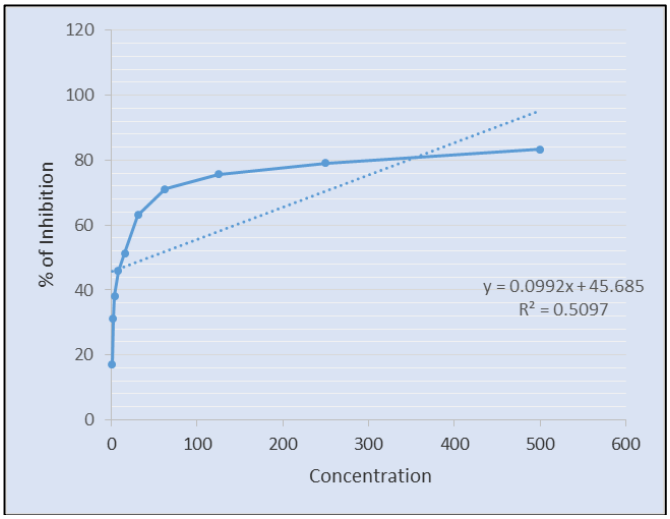

Fig 9: IC_{50} value of AQSF of leaves of *A. bunius*

Discussion

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging [13-14]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule[15]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [16]. Herbal plants considered as good antioxidant since ancient times. Thus, we are trying to investigate the possible development of natural drug alternatives derived from natural sources.

A. bunius belongs to the family euphorbiaceae is a rapidly growing shrubby tree, plays a major role in the medicinal properties. The phytochemical screening of methanolic extract of *A. bunius* leaves has revealed the presence of saponins, phenols, tannins, flavonoids, alkaloids which could be responsible for the versatile medicinal properties or pharmacological actions of this plant part, like anti-diabetic, antioxidant, antiradical, cytotoxicity activity and pesticide agent [17-18]. The fruits of *A. bunius* were evaluated for its antioxidant activity which revealed a positive antioxidant property. After having the result, they got a good amount of antioxidant properties from the experiment of *Antidesma bunius* [19]. Our data revealed that the MeOH extract as well as different solvent soluble fractions of *A. bunius* possess promising antioxidant potential which are attributed to its potent inhibitory activity against DPPH antioxidant scavenging activity. Our data suggested that methanolic extract of *A. bunius* showed potent scavenging activity on *in vitro* antioxidant assays. Among the tested fractions, ethyl acetate and dichloromethane fractions showed potential activity compared to other polar fractions. As shown in Table 1, ethyl acetate fraction exhibited potent DPPH inhibitory activity with IC_{50} value of 136.52 ± 0.03 µg/mL, whereas positive control ascorbic acid showed an IC_{50} value of 43.04 ± 0.01 µg/mL, respectively. Conversely, CH_2Cl_2 fractions also displayed potent DPPH inhibitory activity with respected IC_{50} values of 202.6 ± 0.02 when compared to the positive control ascorbic acid with IC_{50} value of 43.04 ± 0.01 µg/mL respectively. Our study reveals the potent activity of *A. bunius*, establishing them as an alternate source of antioxidant agents.

New therapeutic innovations that are dedicated to prevent oxidative stress in chronic diseases are of interest. Our study revealed the effectiveness of *A. bunius* extract and found potent antioxidant (DPPH), activity. Furthermore, presence of natural constituents like phenol, flavonoid, alkaloid etc. demonstrated antioxidant activity with the potential to prevent inflammation and chronic diseases. Thus, the multifactorial activity of *A. bunius* extracts shows promising antioxidant activity which can be used as a new therapeutic agent for the treatment of oxidative stress as well as chronic diseases.

Conclusion

From the above results it is concluded that *Antidesma bunius* exhibited potential antioxidant activity. *A. bunius* possesses various phytochemical constituents, which may be

responsible for the antioxidant activity. Further work has to be carried out to investigate the active compounds from *Antidesma bunius*.

Conflict of Interest

The authors declare no conflicts of interest.

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