

## IN VITRO ASSESSMENT OF ANTHELMINTIC AND ALPHA-AMYLASE INHIBITION OF *SCHLEICHERA OLEOSA* (LOUR.) OKEN LEAF EXTRACTS

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### ABSTRACT

**Objective:** The present study deals with the effects of *Schleichera oleosa* (Lour.) Oken leaf extracts on helminths and alpha-amylase inhibition. Identification of phytochemicals and physicochemical analysis were also performed.

**Methods:** Different concentrations (25, 50, and 100 mg/ml) of petroleum ether, acetone, chloroform, ethanol, and aqueous extracts of the leaf were used to examine the effects. For the evaluation of *in vitro* anthelmintic activity, several earthworms (*Eisenia fetida*, *Perionyx excavates*, and *Pheretima posthuma*) and nematode (*Ascaridia galli*) were taken, while albendazole was used as a standard drug and Tween 80 (3%) in normal saline (0.9% NaCl) was considered as a control treatment. *In vitro* alpha-amylase inhibition of different extracts (10–100 mg/ml) was done spectrophotometrically by dinitrosalicylic acid - starch azure method.

**Results:** The ethanolic extract showed the maximum presence of phytochemicals among all the extracts, which included alkaloids, tannins, flavonoids, saponin glycosides, phenolic compounds, resins, and amino acids. The outcomes of the determination of physicochemical parameters and fluorescence characters provided the satisfactory results. Significant anthelmintic activity was established by the ethanolic and aqueous extracts of the leaf among all the extracts and the responses, so observed, were dose responsive. Inhibition of alpha-amylase by ethanolic and aqueous extracts was significant with the IC<sub>50</sub> value of 36.63 and 73.94 µg/ml, respectively, when compared to standard acarbose.

**Conclusion:** The ethanolic extract was the more potent candidate for both the effects, and the effect of extract was best against *A. galli*, *P. posthuma*, and *E. fetida* at higher concentration. Isolation and characterization of therapeutic constituents would be the future interest.

**Keywords:** *Schleichera oleosa* (Lour.) Oken, Earthworms, Nematodes, Anthelmintic activity, Alpha-amylase.

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### INTRODUCTION

*Schleichera oleosa* (Lour.) Oken (family: Sapindaceae), is a deciduous tree available throughout Southeast Asia and India, is commonly known as “Kusum” and “Lac tree.” The different parts of this plant have enormous traditional uses to treat different ailments. The leaves are used as fodder for domestic animals [1,2], whereas the bark paste is used for the treatment of malaria and dysentery. In the Himalayan area of Nepal, the fruits are used traditionally as anthelmintic [3], and the seed oil is used for acne, itching, hair growth, and burns; moreover, the whole plant is used as antidiabetic traditionally [4].

Helminthiasis is an extensive infection among the human beings distributed in the tropical region largely due to climate and unhygienic conditions [5,6]. Helminths include flatworms and roundworms or nematodes, which mainly affect the liver, skin, intestine, and liver [7]. To treat ailment like worm infection, usage of herbs is long practice [8-10]. Although the synthetic drugs of benzimidazole group such as albendazole and mebendazole are effective against tapeworms, hookworms, and roundworms [11], their long practice can cause severe side effects such as bone marrow depression and elevated liver enzymes [12].

Diabetes is an important and prior health problem which affected more than 400 million people globally in 2014 and caused 1.5 million deaths in 2012. There is the same alarming condition in Southeast Asia region along with India, and by 2035, the condition will be the worst [13]. Several phytoconstituents of medicinal plants can decrease the postprandial glucose level by inhibiting alpha-amylase [14] as they facilitate the hydrolysis of the starch and its digestion [15,16].

Nevertheless, the plant is used to treat helminthiasis and diabetes; traditionally, the scientific evaluations yet have not been done. Moreover, the phytochemical screening, physicochemical analysis, and fluorescence studies of the plant parts have been evaluated in the present research.

To evaluate the *in vitro* anthelmintic activity of different plant extracts, different earthworms such as *Eisenia fetida* (Family: Lumbricidae), *Perionyx excavates* (Family: Megascolecidae), and *Pheretima posthuma* (Family: Annelida) and nematodes (*Ascaridia galli*) have been selected in the present study for their anatomical and physiological uniformities with the intestinal worms.

### MATERIALS AND METHODS

#### Plant material

The leaves of *S. oleosa* (Lour.) Oken were collected from the forest area of Nichlaul, Maharajganj district, Uttar Pradesh, in the flowering season of February and March 2017. The clean leaves were air dried for 3 months. Indian Council of Agricultural Research-Kamla Nehru Krishi Vigyan Kendra, Sultanpur, Uttar Pradesh, India (accession no. 02/2017), authenticated the plant parts.

#### Preparation of crude extracts

The shade dried coarsely powdered of leaves of the plant was subjected for successive solvent extraction according to the polarity. The different solvent extracts were petroleum ether (SEPE), chloroform (SECE), acetone (SEAcE), ethanol (SEEE), and water (SEAE). The cold maceration process was employed for the aqueous extract, and the continuous hot percolation process was employed for the others. After

the extraction, the solvents were removed by distillation, and residues were preserved in the refrigerator at 2–8°C to use in the experiments.

### Preliminary phytochemical screening

To confirm the presence or absence of different chemical constituents such as alkaloids, saponin glycosides, tannins, flavonoids, phenolic compounds, steroids, proteins, and amino acids, the preliminary phytochemical studies with different solvent extracts were performed according to the standard procedure [17].

### Physicochemical analysis

The WHO recommended procedure was employed to evaluate different physicochemical parameters such as total ash, acid-insoluble ash and water-soluble ash values, extractive values with different solvents, loss on drying, and foreign matter [18].

### Fluorescence study

The leaf powder and treated with different solvents were subjected for the fluorescence study. The observations were made under daylight, short UV light at 254 nm, and long UV light at 365 nm [19].

### Collection and authentication of worms

Different earthworms such as *E. fetida*, *P. excavates*, and *P. posthuma* were collected from moist soil and waterlogged areas. The domestic chicken (*Gallus gallus*) was freshly slaughtered to obtain the nematodes (*A. galli*). All the worms were washed with saline water to remove foreign and soil particles. Department of Zoology, HRP College Khalilabad, Uttar Pradesh (Accession no: 09-Zoo-17), authenticated all the worms.

### In vitro anthelmintic activity

*In vitro* anthelmintic activity was performed with all the extracts by taking different earthworms and nematodes according to the previous studies with slight modifications [5,20,21]. Almost equal sizes of *P. posthuma*, *E. fetida*, *P. excavates*, and *A. galli* were chosen (each group, n=6) for the activity. Similar studies using these models have been reported earlier [22-24].

The standard drug, albendazole, and all the extracts (dissolved in 3% Tween 80 in normal saline) were prepared in 25, 50, and 100 mg/ml concentrations, while Tween 80 (3%) in normal saline (0.9% NaCl) was considered as control treatment and kept in separate Petri dishes, containing the worms. Paralysis time (PT in min) was noted while there was no motility of the worms even after extensive shaking and death time (DT in min) was recorded when there was a loss of motility even after dipping the worms in the warm water of 50°C.

The statistical evaluation was performed by one-way ANOVA; the results were expressed as  $\pm$ standard deviation (SD) using Graph Pad Prism 7.4 (n=6) and p<0.05 was considered statistically significant.

### In vitro alpha-amylase inhibition activity

The assay was performed following the standard methods with minor modifications [25,26]. 0.5% w/v starch solution was prepared by mixing 250 mg starch azure in 50 ml of 20 mM sodium phosphate buffer (pH 6.9) with 6.7 mM NaCl at pH 6.5°C for 15 min. Plant extracts were prepared in different concentrations from 10 to 100 mg/ml by dissolving in dimethyl sulfoxide (DMSO). 1 ml of each plant extract was mixed with 1 ml of enzyme solution, which was prepared by mixing 0.0253 g of alpha-amylase in 100 ml cold distilled water. To the 1 ml of this above solution, 1 ml of starch solution was added, and the tube was incubated for 5 min at 25°C. The colorimetric reagent was prepared by mixing 12 g sodium potassium tartrate tetrahydrate in 2 M NaOH (8 ml) and 96 mM 3,5-dinitrosalicylic acid (20 ml), which was added to the above solution and placed on a water bath at 80°C for 15 min followed by cooling and adding of 9 ml distilled water.

Acarbose of different concentrations (10–100 mg/ml) was referred to standard drug. Blanks for each extract were prepared by omitting

enzyme solution by phosphate buffer (pH 6.9) and color reagent was added before the starch solution. Controls were prepared by taking 1 ml DMSO instead of plant extract, the rest of the procedure was performed same as with plant extracts. The absorbance of standard, samples, and blanks was measured at 540 nm in UV spectrophotometer. The enzyme inhibitory activity was calculated by following formula:

$$\text{Alpha - amylase inhibitory activity (\%)} = \frac{\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}}{\Delta A_{\text{Control}}} \times 100$$

Where, A=Absorbance

$\Delta A_{\text{Control}} = A_{\text{of Test}} - A_{\text{of Blank}}$

$\Delta A_{\text{Sample}} = A_{\text{of Test}} - A_{\text{of Blank}}$

The statistical evaluation was performed by linear regression analysis; the results were expressed as  $\pm$ SD using Graph Pad Prism 7.4 (n=3). The results were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests and p<0.0001 was considered statistically significant.

## RESULTS AND DISCUSSION

### Preliminary phytochemical screening

The preliminary phytochemical screening of the plant leaf extracts affirmed the maximum presence of chemical constituents in SEEE and SEAE while comparing to others and the data have been tabulated in Table 1, which showed the presence of alkaloids, tannins, flavonoids, saponin glycosides, phenolic compounds, resins, and amino acids in SEEE.

The presence of these phytochemicals has a direct effect on helminthiasis. Alkaloid causes paralysis of helminths by interfering with local homeostasis which has the vital role in the development of worms [27]. Saponin glycosides, found in the wide variety of higher plants, can cause the damage integument and subsequent paralysis of the worms [6]. The previous literature described the presence of tannins in plant extracts can cause damage of cuticles of helminths by binding with the glycoproteins present on it [28,29]. Phenolic compounds and flavonoids are involved in energy generation process, damage in cuticle, and ultimately cause the paralysis and death of the worms [30].

Moreover, alkaloids, tannins, phenolic compounds, and flavonoids accelerate insulin secretion and reduce the absorption of glucose in intestine [31]. All these phytochemicals, as reported in literature, possess alpha-amylase inhibition activity [32].

### Physicochemical analysis and fluorescence study

Plant leaf powder was subjected for the evaluation of different physicochemical parameters and fluorescence study, which represented the characteristic properties of the leaf of *S. oleosa* (Lour.) Oken. The results of the above studies were given in Tables 2 and 3, respectively.

Ash values were found to be the highest (6.21%) while water-insoluble ash was the lowest (3.22%). Extractive values were found mostly in the aqueous extract (27.3%), followed by ethanolic extract (26.1%), ethyl acetate, chloroform, acetone, and hexane extract.

**Table 1: Preliminary phytochemical screening of *Schleichera oleosa* (Lour.) Oken leaf**

Phytochemicals	SEPE	SECE	SEAcE	SEEE	SEAE
Alkaloids	-	-	-	+	-
Saponin glycosides	-	-	-	+	-
Tannins	-	+	+	+	+
Phenolic compounds	+	+	+	+	+
Flavonoids	-	-	-	+	+
Resins	-	-	-	+	-
Protein and amino acids	-	-	+	+	-

+ indicates "presence" and - indicates "absence" of phytochemicals in the test.

SEPE: Solvent extracts of petroleum ether, SECE: Solvent extracts of chloroform,

SEAcE: Solvent extracts of acetone, SEEE: Solvent extracts of ethanol,

SEAE: Solvent extracts of water

**In vitro anthelmintic activity**

For the support of the folkloric use of *S. oleosa* (Lour.) Oken as anthelmintic [3], an *in vitro* assay was performed by noting down the PT and lethal time in minutes of all extracts. Due to the anatomical and physiological resemblance with the human intestinal worms and parasites, different earthworms and nematodes were selected for the activity. In the observed results, the earthworms and nematodes likely *E. fetida*, *P. excavates*, *P. posthuma*, and *A. galli* were denoted as EP, PE, PP, and AG, respectively. The effect on all the worms of standard drug albendazole was significant and dose dependent which supports the reported related literature [33,34].

At the concentration of 100 mg/ml, the standard drug was more effective against all worms. The results were presented in graphical form; Figs. 1-3 described the effect of plant extract on EP, PE, PP, and AG at different concentrations. There was no effect on the worms in control treatment as the worms were healthy throughout the experiment.

Among all the extracts, SEEE is considered more effective against all the earthworms and nematodes while comparing with others. Moreover, SEEE showed greater effect on *A. galli* (DT: 41.03±4.21 minutes at 100 mg/ml) with the increasing dose than the other earthworms, and among the earthworms, the extract has greater significant effect on *E. fetida* (DT: 56.1±2.41 min at 100 mg/ml) and *P. posthuma* (DT: 57.1±5.48 min at 100 mg/ml) while comparing to *P. excavates* (DT: 62.31±3.44 min at 100 mg/ml). It has been reported in earlier studies that the ethanolic extract of *Clerodendrum viscosum* was more effective against *A. galli* than *P. posthuma* in 50, 100, and 200 mg/ml dose [35]. Likewise, at lower concentration (10 and 50 mg/ml), *Baliospermum montanum* Muell. Arg. was proved as potent anthelmintics against *A. galli* than *P. posthuma*. At the higher concentration, the plant was equally potent against them. In addition, this study has reported that the ethanolic extract was more potent than aqueous extract [36].

**Table 2: Physicochemical analysis of *Schleichera oleosa* (Lour.) Oken leaf**

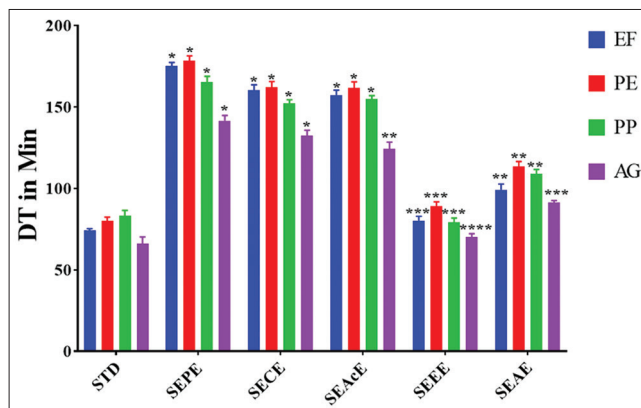
Sr. No	Parameters	Value
1.	Ash values	
	Total ash	6.21% w/w
	Acid-insoluble ash	4.72% w/w
	Water-soluble ash	3.22% w/w
2.	Extractive values with organic solvent	
	Hexane	8.2% w/w
	Chloroform	12.3% w/w
	Acetone	9.7% w/w
	Ethyl acetate	17.2% w/w
	Ethanol	26.1% w/w
	Water	27.3% w/w
3.	Loss on drying	5.7±0.3 mg/g
4.	Foreign matter	2.15% w/w

**Table 3: Fluorescence analysis of *Schleichera oleosa* (Lour.) Oken leaf**

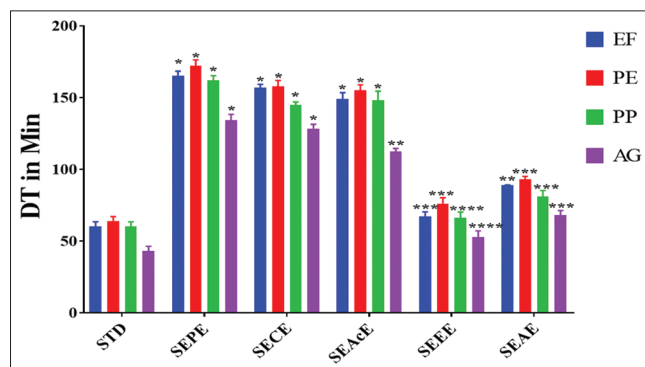
Sr. No	Reagents	Daylight	Short UV	Lower UV
1.	Leaf Powder (LP)	DG	Bl	YG
2.	LP+1 N HCl	GBr	Br	DBr
3.	LP+HCl (50%)	GBl	DBr	DG
4.	LP+1 N NaOH	YG	DBr	GBl
5.	LP+H <sub>2</sub> SO <sub>4</sub> (50%)	GBr	DBl	RBr
6.	LP+HNO <sub>3</sub> (50%)	YG	LBl	GBl
7.	LP+NH <sub>3</sub>	LG	RG	DG
8.	LP+GAA	GBl	LBr	RBr
9.	LP+Ethanol	GBr	GBr	DG
10.	LP+KOH (50%)	YG	LG	DG

LP: Leaf powder, GAA: Glacial acetic acid, L: Light, D: Dark, G: Green, Br: Brown, Bl: Black, BrR: Brownish-red, GBr: Greenish-brown, GBl: Greenish-black, RBr: Reddish-brown, YG: Yellowish-green, YBr: Yellowish-brown

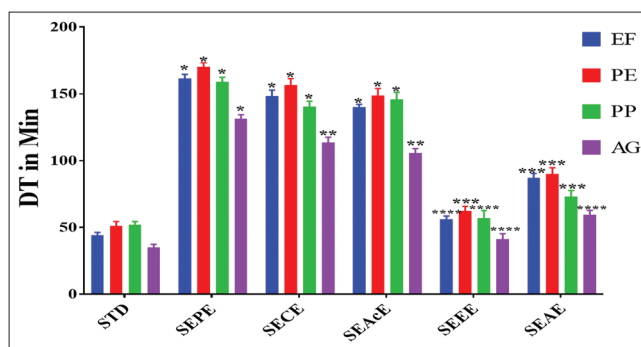
The present study also confirmed that SEEE was more effective than SEAE. In the case of SEAE, the effectiveness was more against the nematode (DT: 59.21±3.56 min at 100 mg/ml), and almost equal against the earthworms. In the earlier literature, the significant anthelmintic activity of chloroform extract of other plants against earthworms has been reported [37,38]. Tannins containing acetone extract were evaluated for the anthelmintic activity against *Haemonchus contortus* egg hatching in the previous literature which was considered as potent anthelmintics [39]. Similarly, this present paper also described the



**Fig. 1: The anthelmintic activity of *Schleichera oleosa* (Lour.) Oken leaf extracts at 25 mg/ml. All values represent mean±standard deviation; n=6 in each group; p<0.05 was considered statistically significant**



**Fig. 2: The anthelmintic activity of *Schleichera oleosa* (Lour.) Oken leaf extracts at 50 mg/ml. All values represent mean±standard deviation; n=6 in each group; p<0.05 was considered statistically significant**



**Fig. 3: The anthelmintic activity of *Schleichera oleosa* (Lour.) Oken leaf extracts at 100mg/ml. All values represent mean±standard deviation; n=6 in each group; p<0.05 was considered statistically significant**

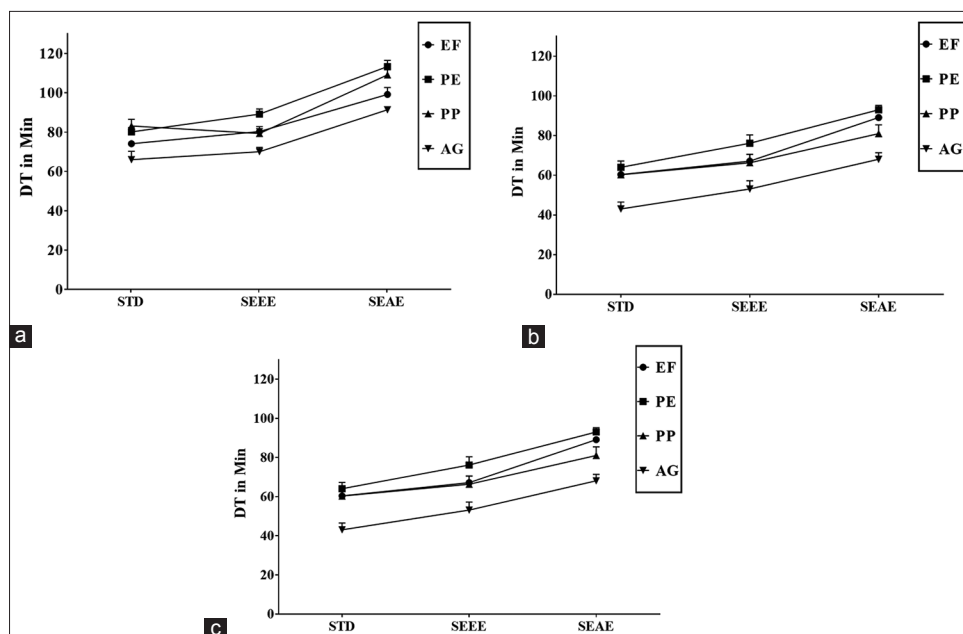


Fig. 4: The comparative anthelmintic activity of solvent extracts of ethanol and water with the standard drug (albendazole) at (a) 25 mg/ml; (b) 50 mg/ml; and (c) 100 mg/ml (n=6; all values represent mean±standard deviation;  $p < 0.05$  was considered statistically significant)

significant effect of the SECE and SEAcE on *A. galli* (DT:  $113.45 \pm 4.11$  min and  $105.78 \pm 3.25$  min at 100 mg/ml, respectively) but not as significant as the ethanolic one. The results also exhibited the poor effect of SEPE extract had a very little effect on the worms.

The results exhibited clearly the effects of all the extracts were dose dependent; at 25 mg/ml, SEEE was more effective against all the worms and SEAE was found effective against *A. galli* (Fig. 4a). In Fig. 4b, it has been found that at 50 mg/ml dose SEEE showed best result against *A. galli* than *P. posthuma*. At the higher concentration, 100 mg/ml, the response of SEEE and SEAE was most potent against the nematodes (Fig. 4c).

#### In vitro alpha-amylase inhibition activity

Inhibitor of alpha-amylase enzyme is a better oral hypoglycemic agent as they control diabetes by interfering with the absorption of glucose [40]. The present evaluation of *in vitro* alpha-amylase inhibition was done by dinitrosalicylic acid - starch azure method, for the support of its traditional use as an antidiabetic plant. At the range of 10–100 mg/ml, standard drug (acarbose) exhibited alpha-amylase inhibition  $19.25 \pm 0.06$ – $62.38 \pm 0.05$  ( $IC_{50}$  value 78.56), whereas SEEE and SEAE showed the inhibition in the same concentration range from  $32.40 \pm 0.95$  to  $83.51 \pm 1.0$  ( $IC_{50}$  value 36.63) and  $19.62 \pm 0.90$  to  $62.30 \pm 2.03$  ( $IC_{50}$  value 73.94), respectively. Similar studies of the plant *S. oleosa* (Lour.) Oken showed similar effect; at the lower concentration (1–50 mg/ml), ethanolic and aqueous extract showed better inhibition activity while comparing to other extracts of ethyl acetate, pet ether, and chloroform [41]. The present study revealed that at higher concentration (60–100 mg/ml) also ethanolic and aqueous extracts were more significant than others. Figs. 5 and 6 represented the comparison of alpha-amylase inhibition activity and the  $IC_{50}$  values of different extracts while compared to acarbose, respectively. At 100  $\mu$ g/ml, the enzyme inhibition of the other extracts: SEPE ( $19.53 \pm 0.16$ ), SECE ( $33.39 \pm 1.31$ ), and SEAcE ( $45.61 \pm 2.08$ ) proved SEEE and SEAE more potent.

#### CONCLUSION

The present study unveiled the plant *S. oleosa* (Lour.) Oken as a potent anthelmintics and antidiabetics supporting the traditional beliefs. The

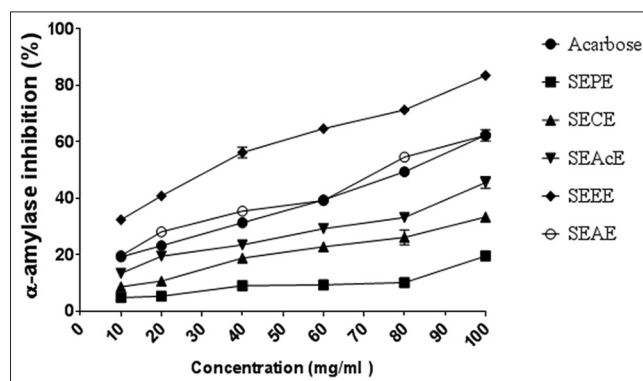


Fig. 5: Alpha-amylase inhibition of *Schleicheria oleosa* (Lour.) Oken leaf extracts compared to standard (acarbose) (n=triplicate; all values express mean  $\pm$  standard deviation)

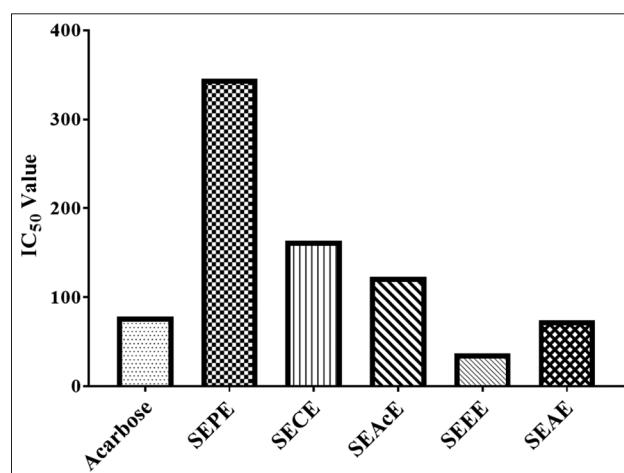


Fig. 6:  $IC_{50}$  values of *Schleicheria oleosa* (Lour.) Oken leaf extracts compared to standard (acarbose)

ethanolic and aqueous extract of the plant was manifested as more effective against helminthiasis, especially against the nematodes, as well as showed better alpha-amylase inhibition activity. The identification of the secondary metabolites of the plant parts to explore the constituents responsible for the activity and molecular level mechanism will be the future interest.

#### AUTHORS' CONTRIBUTION

Goswami Shambaditya: Performs all experimental works, designing of results, and manuscript preparations. Singh Ravindra Pal is a research advisor and performs statistical analysis and manuscript editing. Both the authors read and approved the final manuscript.

#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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