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EVALUATION OF *IN VITRO* ANTIOXIDANT, ANTIDIABETIC AND ANTIMICROBIAL ACTIVITIES OF PETALS OF *BOMBAX CEIBA* L.

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Abstract

Bombax ceiba, endemic to semal, is commonly known species. Lots of study had done on this species due to the fact that this plant is widely used in traditional and alternative medicines in India. In this study, Soxhlet extraction on the basis of polarity by different solvent like petroleum ether (P.E), chloroform (C.H), ethyl acetate (E.A), ethanol (E.L) and water (W.R) and their biological activities like free radical scavenging activity, total phenolic content, antidiabetic and antimicrobial have done. Free radical scavenging activity was determined according to the DPPH and FRAP method. Total phenol content was determined by the Folin-Ciocalteu reaction, antidiabetic activity was done by using α-glucosidase and α-amylase method and antimicrobial assay was done by using disk diffusion method. Ethyl acetate extract of *B. ceiba* L. was found to have highest antioxidant and antidiabetic activity with IC_{50} 80.05µg/ml and 165-180µg/ml respectively. This effect was found to be related to the total phenolic content of ethyl acetate extracts. Antimicrobial activity of petals was also highest inhibition in ethyl acetate extract with MIC 1.87. Petals of *B. ceiba* is thought to be a natural source of antioxidants, antidiabetic and antimicrobial.

Key words: *Bombax ceiba* L., biological activities antioxidant, antidiabetic and antimicrobial.

Introduction

A number of herbal drugs are advocated in the traditional Ayurvedic literature for the improvement of overall healthcare system. *Bombax ceiba Linn* also known as Semal Musli are used traditionally in Indian subcontinent as sexual stimulant. Its juice is considered nutritive and restorative tonic. Among the well-known medicinal plants *Bombax ceiba* L. is one of the important medicinal plant belongs to the family Bombacaceae play a significant role in protecting the well-being population. *Bombax ceiba* L. is widely distributed and cultivated in temperate Asia, possesses a strong ethnobotanical background, and extensively used as a famous folk medicine in the treatment of a wide range of diseases (Joshi *et al*., 2014; Bhargava *et al*., 2012; Robyns *et al*., 1963). *Bombax ceiba* L. is a diploid deciduous tree, is widely distributed in temperate and tropical regions, such as Southeast Asia, Africa and Australia native to South Asia, Southeast Asia, southern China and northern Australia (Gewali *et al*., 2014; Grierson *et al*., 1983; Hnatiuk, 1990). *Bombax ceiba* L. used in multipurpose like- It provides food, fodder, fiber, fuel, medicine and many other ecological benefits (Singh and Panda, 2005). It is also an important component of tropical dry deciduous forest ecosystems (Rameshwar *et al*., 2014; Panda *et al*., 2011). The objective of this work was to investigate the antioxidant, antidiabetic and antimicrobial activities of the polar and nonpolar extracts of petals of *Bombax ceiba* L. flower by *in vitro* methods.

Materials and Methods

Foline-Ciocalteu (FC) reagent, sodium carbonate anhydrous, gallic acid (G.A), ascorbic acid (A.A), sodium nitrite, sodium hydroxide, aluminum chloride anhydrous, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, ferric chloride anhydrous, ascorbic acid, and catechin were purchasedfrom Sigma-Aldrich GmbH (Sternheim, Germany). Sulfuric acid and potassium-hexacyanoferrate were obtained from Merck (Darmstadt, Germany). Anhydrous monobasic potassium phosphate was purchased from Fisher Chemicals (Fair Lawn, NJ, USA),whereas HPLC-grade methanol, ethanol, and acetone were supplied by Sigma-Aldrich.

Sample collection and preparation

B. ceiba was collected from campus of Gurukul Kangri Vishwavidyalaya, Haridwar (daytime air temperature (12-17.2°C) of Uttarakhand of India in the month of January 2017 and authenticated from Botanical survey of India (BSI) Dehradun (Voucher specimen number 117964 08/2017). Flower petals were separated and dried for 15-20 days under shade until petals seems to be ready for grinding and stored at room temperature, were subjected to grinding in a laboratory grinder and stored at 4°C (Ali *et al*., 2006).

Preparation of extract

Dry powdered material of petals (200 g) were packed into a Soxhlet apparatus and extracted with 800ml of each solvent successively in increasing order of polarity. The extracts were filtered through Whatman filter paper No. 1 and the filtrate was concentrated under reduced pressure at 40°C. The extracts were dried, weighed and stored at 4ºC storage vials for experimental use (Shen and Shao, 2005).

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Total phenolic content

The total phenolic content of the extract was determined by the Folin-Ciocalteu method (Yu *et al*., 2011). Briefly, 200ml of crude extract (1 mg/mL) were made up to 3ml with distilled water, mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark and absorbance was measured at 650nm. Thetotal phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per gm dry weight.

Antioxidant Properties

2,2-Diphenyl-1-picryl-hydrazyl assay

The antioxidant activity of the extract was determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, as described earlier with some modifications (Naik *et al*., 2011). Briefly, 2ml of each extract (5-1000 mg/mL) were mixed with 3.8ml DPPH solution and incubated in the dark at room temperature for 1 h. The absorbance of the mixturewas then measured at 517 nm. Ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from:

$$\text{DPPH scavenging effect} = \frac{\text{Control absorbance-Sample absorbance}}{\text{Control absorbance}} \times 100$$

Ferric reducing power assay

Ferric reducing or antioxidant power was determined as described earlier (Yu *et al*., 2011). Briefly, 100µL of the extract (100-500 µg/ml) were mixed with 2.5ml of 200 mmol/L phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide and incubated at 50°C for 20 min. Then, 2.5ml of 10% trichloroacetic acid were added and the tubes were centrifuged at 10,000 rpm for 10 min. Then, 5ml of the upper layer were mixed with 5.0ml distilled water and 1ml of 0.1% ferric chloride and the absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive-control.

Antidiabetic Activity

Inhibition of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/vmaltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in Boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method (Watanabe *et al*., 1997).

$$\text{Alpha glucosidases inhibition} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

Inhibition of alpha amylase enzyme

A total of 500 µl of test samples and standard drug (10-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3,5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle (Ali *et al*., 2006).

Antibacterial activity

Antimicrobial activity was tested in both Gram-negative and Gram-positive bacteria obtained from the Departmentof microbiology, Gurukul Kangri Vishwavidyalaya, Haridwar, India. The strains were maintained by periodic subculture on nutrient agar and preserved at 4°C prior to use. They were grown overnight in 10ml broth at37°C, which was then centrifuged at 150 rpm. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method (Panda *et al*., 2011; Naik *et al*., 2011). Serial dilutions of the stock solutions of the crude extract in both medium were prepared on a microliter plate and microbial suspensions were added to the microwells at 5×105 micro-organisms/ml. The microliter plates were then incubated at 37°C for 24 h. Activity was recorded as blue coloration in the wells after addition of resazurin. MIC's were determined as the lowest concentrations that prevented visible growth. Streptomycin was used as apositive control. Each assay was repeated three times.

Results

After the complete extraction of flower petals raw material by different solvents found different yield, nature and color of plant extract. Highest yield found in water (22.194 gm) extract and least in chloroform (0.892 gm). The total phenolic contents of the extracts are shown in Table 1. The total polyphenol content of *B. ceiba* petals were highest in ethyl acetate and ethanol extract and the minimum in non-polar petroleum ether extracts. According to reported literature, polar solvents like ethyl acetate and ethanol are most promising solvents for the extraction of phenolic and flavonoid content.

Extracts of *B. Ceiba* petals were analyzed against DPPH synthetic radical. Hexane extract had the lowest anti-radical activity, while chloroform and water had moderate activity and polar solvents ethyl acetate and 99% ethanol were the most potent. Thus, phenolic compounds from ethyl acetate and ethanol extracts of *B. ceiba* were more efficient antioxidants than non polar solvent extracts. The FRAP assay is determined by the ferric reducing ability of petals crude extracts (Table 2). The successive Soxhlet ethanol extract (7075.41 mol Fe(II) equiv./g) showed higher ferric reducing ability compared to ascorbic acid (6341.84 µmol -Fe(II) equiv./g). The non-polar extracts showed less ferric reducing ability 5404.11 mol Fe(II) equiv./gand 48631 mol Fe(II) equiv./g, respectively.

The CD methanol extract revealed a significant inhibitory action on alpha glucosidase enzyme. The percentage inhibition at 100-1000 µg/ml concentrations of

CD extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 85.71±0.918 to 30.78±0.4855 for highest concentration to the lowest concentration of 100 µg/ml. The concentration required for 50% inhibition (IC_{50}) was found to be 402.23±10.14 µg/ml whereas the α -glucosidase inhibitory activity of positive control acarbose produced percentage of 40.73±1.39 for 100 µg/ml and 91.58±1.39 for 1000 µg/ml. The IC_{50} value of standard drug acarbose against alpha glucosidase was found to be 325.50 ± 4.7 µg/ml (Table 3).

There was a dose dependent increase in percentage inhibitory activity against alpha amylase enzyme. At a concentration 100 µg/ml of extract showed a percentage inhibition 23.62±0.2454 and for 1000 µg/ml it was 61.31±0.3729. The extract gave an IC_{50} value of 686.94±3.98µg/ml. The IC_{50} value of standard drug acarbose was found to be 325.50±4.7 µg/ml (Table 1). As the initial results showed that *B. ceiba* at the flowering stage had a higher phenol content and greater antioxidant activity than plants in the vegetative stage, the antimicrobial activity of this plant was evaluated only at flowering. Table 4 shows the antibacterial activity of extracts of *L. delicatulum* shoots. Hexane and aqueous extracts had no antimicrobial activity, methanol and acetone extracts had moderate activity against *Staphylococcus aureus*, *P. aeruginosa*, *L. monocytogenes* and *M. luteus*, while ethanol extracts had good inhibitory activity against *Salmonella*, *E. coli*, *S. aureus* and *L. monocytogenes* and moderate activity against *Enterococcus faecium*, *Pseudomonas* and *M. luteus*. The antibacterial potential was dose-dependent.

Discussion

After the complete extraction of plantraw material by different solvents found different yield, nature and colour of plant extract. Highest yield found in water extract and least in chloroform (Graph 1). DPPH radical is a stable organic fre radical with an absorption band at 512 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Zheng and Wang, 2001). Figure 1 shows the DPPH scavenging activities of the extracts in a concentration dependent manner. The extract obtained by 100% ethyl acetate yielded the highest DPPH radical scavenging activity at concentrations ranging from 25 mg/mL to 200 mg/ml. However, at concentrations ranging from 200 mg/ml to 350 mg/ ml, its DPPH radical scavenging activity is not significantly different from those of the other extracts. All extracts obtained by using a pure organic solvent and ethyl acetate gave stronger radical scavenging capacity than that of the other extracts (Figure 1 & Table 3).

The FRAP assay is determined by the ferric reducing ability of leaves crude extracts. Antioxidant potentialisvisually by noticeable coloration from transparent or yellow to purple. The successive Soxhlet ethyl acetate extract showed higher ferric reducing ability compared to ascorbic acid. The petroleum ether and water extract showed least ferric reducing ability (Table 2). In vitro, antidiabetic activity of *B. ceiba* was also conducted based on two biochemical assays. There could be some problems in using animals in experimental in-vivo research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. In α-amylase method, inhibition effect by phyto-constituents responsible for antidiabetic potential of each extract based on change in color intensity was monitored at 540 nm. IC_{50} value obtained for chloroform extract was lower than IC_{50} values of other extracts (Table 3). Antidiabetic potential by α-glucosidase and α-amylase was measured with the help of P-nitrophenyl-α-D-glucopyranoside (NPG) and tris- buffer and 50% inhibition of chloroform extract was reached at a concentration much less than other extracts (Figure 2 & 3).

Five extract of plant were investigated to evaluate their antibacterial activity against food poisoning bacteria including two strains of Gram positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) and two strains of Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using disc diffusion method. Evaluation of antibacterial activity of *B. ceiba* extracts was recorded in Table 4. The results revealed that ethyl acetate extract were potentially effective in suppressing microbial growth of food poisoning bacteria with variable potency of all tested pathogenic bacteria at concentrationof 25 mg/ml while ethanol extract was less effective against *S. aureus* and *B. ceiba* and water extract was unaffected against all becteria. Other plant extracts showed variable antimicrobial activity against different bacterial strains. Results of antimicrobial activity of the five extracts can suggested that *S. aureus* was the most resistant strain to ethyl acetate extracts followed by *P. aeruginosa* while *E. coli* was the most susceptible strains to the all extracts of plant respectively. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) against the bacterial strains (Table 5).

Conclusion

The present study revealed that different types of extraction methods had a big influence on the antioxidant, antidiabetic and anti-microbial properties of obtained extracts. These results showed that *B. ceiba* could be a potential natural source of antioxidant, phenolic, antidiabetic and antimicrobial and could have greater importance as therapeutic agent in preventing or slowing oxidative stress and inflammation related disorders. Further studies are currently underway to assess the in-vitro biological activities and to identify the active component responsible for their antioxidant andanti-microbial properties.

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Table 1: Total Phenolic contents of *B. ceiba* petal extract

S. No.	Extract	Total Phenolic Content (g ACE/100gm dw)*
1	Petroleum ether	0.72
2	Chloroform	19.26
3	Ethyl acetate	40.45
4	Ethanol	17.421
5	Water	32.91

Table 2: FRAP antioxidant free radical scavenging activity

S. No.	Extract (µM/ml)	FRAP Value
1	P.E.	0.800
2	C.H.	1.200
3	E.A.	1.511
4	E.L.	0.622
5	W.R.	0.955
6	A.A.	2.000

Table 3: $IC_{50\ Value}$ of Antioxidant and Antidiabetic activities of different *Bombax ceiba* L. extracts using Ascorbic acid and Acarbose respectively

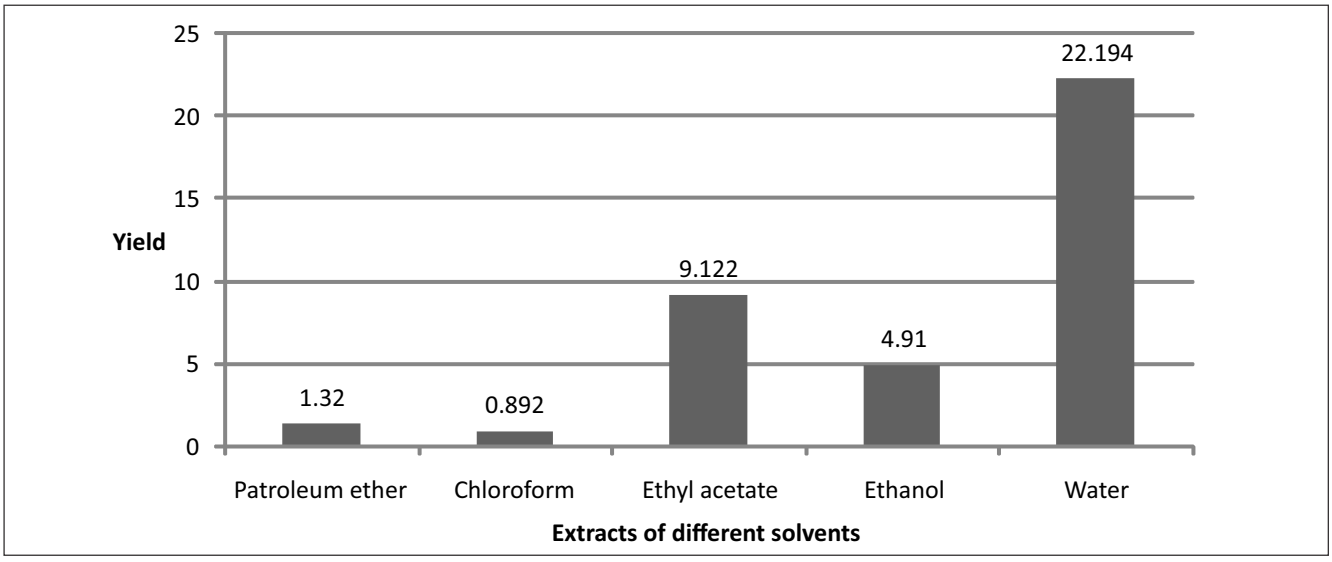
S. No.	Plant extracts	IC_{50}(µg/ml) DPPH antioxidant assay	IC_{50}(µg/ml) Alpha-glucosidase assay	IC_{50}(µg/ml) Alpha-amylase assay
1	Petroleum ether	1682.25	1465.25	1590.80
2	Chloroform	780.10	889.48	905.26
3	Ethyl acetate	80.05	165.15	180.25
4	Ethanol	480.40	210.74	255.14
5	Water	70.15	244.74	265.42
6	Ascorbic acid	35.25	-	-
7	Acarbose	-	60.45	70.24

Table 4: Zone of inhibition (in mm) of bacterial species in *Bombax ceiba* L. petal extracts and Erythromycin

Extract/ standard	Concentration (mg/ml)	Inhibition zone (mm)					
		Gram (+ve) pathogenic bacteria		Gram (-ve) pathogenic bacteria			Fungal Pathogen
		S. aureus	*L monocytogenes*	*P. aeruginosa*	*V. Cholerae*	*S. typhi*	*C. albicans*
P.E.	25	10	10	9	9	10	10
	50	14	13	10	10	10	15
	100	19	17	12	11	13	20
C.H.L.	25	10	10	11	11	14	10
	50	13	15	12	12	15	14
	100	15	19	15	14	16	17
E.T.C.	25	20	18	17	12	11	20
	50	26	23	22	16	14	26
	100	29	27	25	20	15	30
A.L.C.	25	16	13	11	13	11	15
	50	20	17	15	14	13	18
	100	25	21	20	15	14	20
H_20	25	7	8	9	0	0	6
	50	11	10	10	0	11	9
	100	12	13	11	0	13	12

Table 5: Minimum Inhibitory Concentration (MIC) of test pathogens in *Bombax ceiba* L. petalextracts and Erythromycin

Pathogens/Extract	P.E.	C.H.L.	E.T.C.	A.L.C.	H_20
P. aeruginosa	6.25	3.75	1.87	3.75	12.5
V. Cholerae	6.25	3.75	3.75	3.75	12.5
S. typhi	6.25	1.87	1.87	3.75	12.5
C. albicans	6.25	3.75	1.87	1.87	12.5
L. monocytogenes	6.25	3.75	1.87	3.75	12.5
S. aureus	6.25	3.75	1.87	1.87	12.5



Graph 1: Showing yield of extract by different solvents

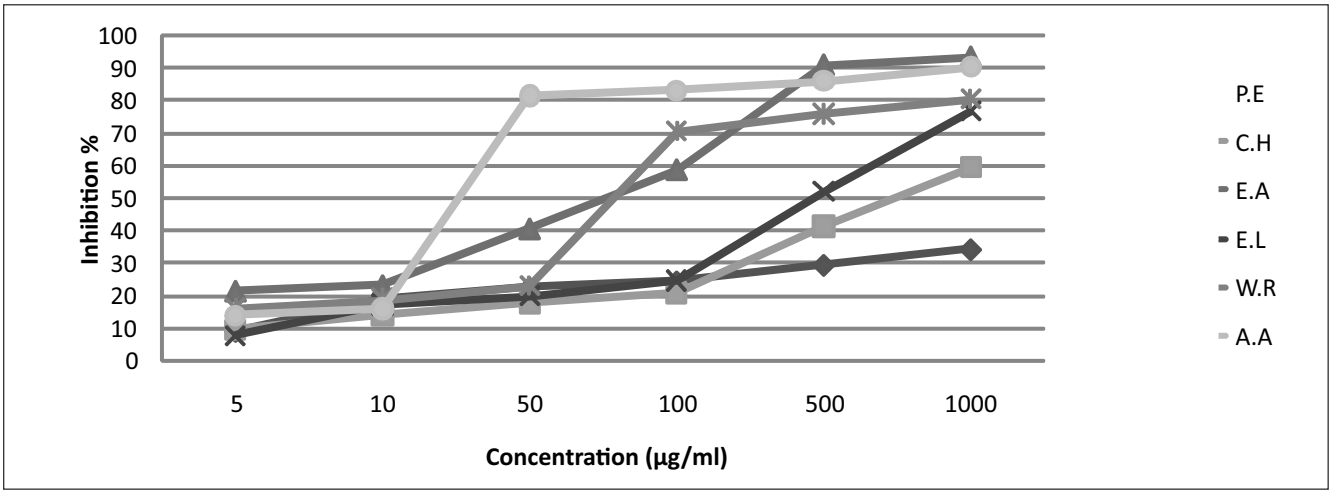


Figure 1: Inhibition for Antioxidant assay of *B. ceiba* petals

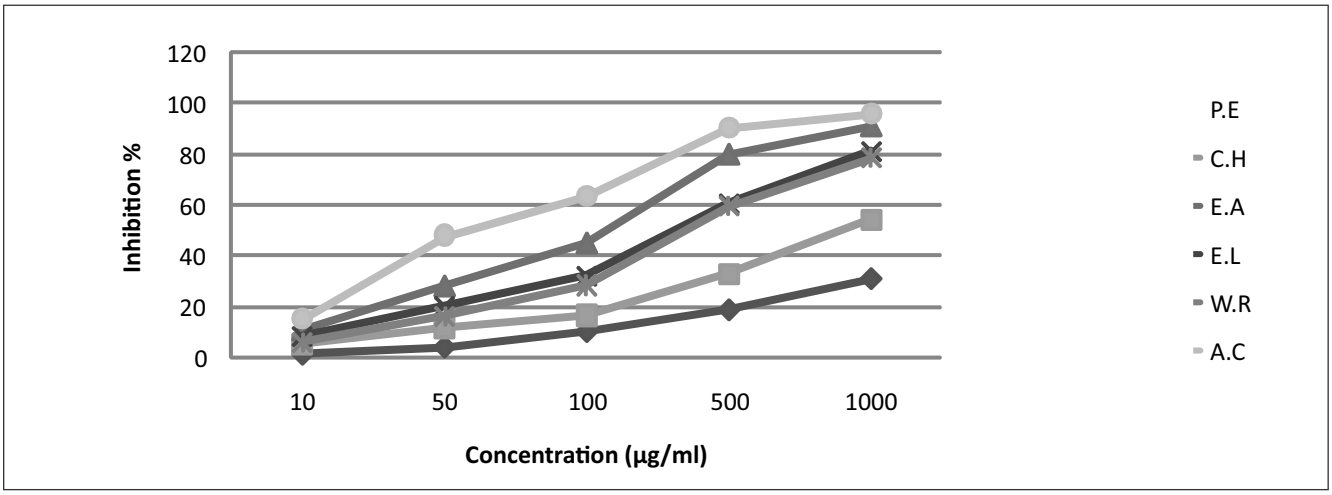


Figure 2: Inhibition for Anti-diabetic assay by alpha glucosidase of *Bombax ceiba* L. petals

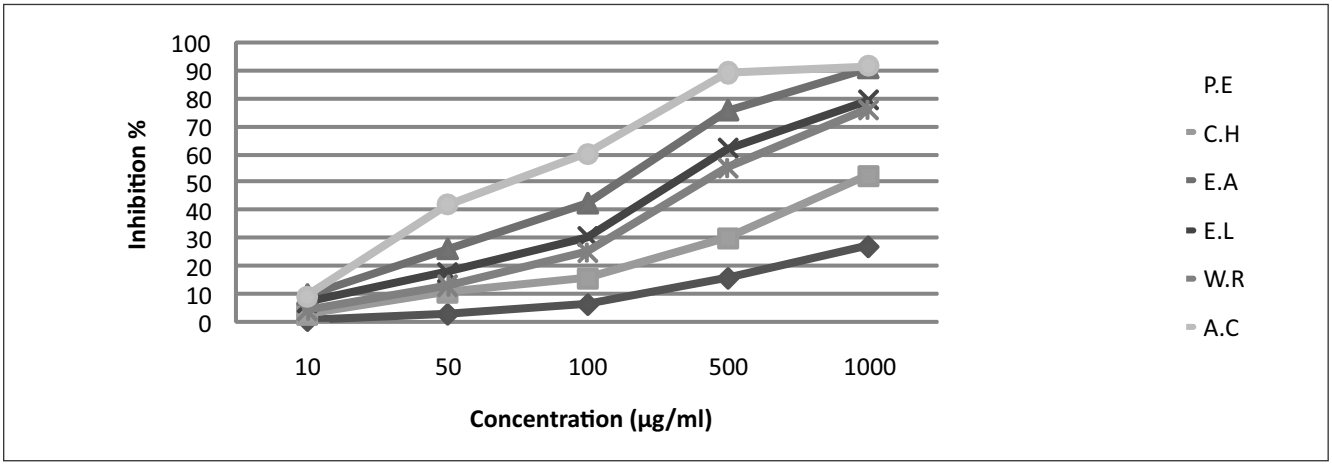


Figure 3: Inhibition for Anti-diabetic assay by alpha amylase of *Bombax ceiba* L. petals