STUDY OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *BOMBAX CIEBA* FLOWER EXTRACT

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Abstract: Plants have been an important source of medicines since the beginning of cultivation. There is a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Bombax cieba* Linn. (Bombacaceae) is a tall tree buttressed at the base that is widely distributed throughout India. The present work was decided to evaluate the in-vitro antimicrobial and antioxidant activity of crude extract of Bombax cieba. Petroleum ether, Chloroform and Methanol extracts of *B. cieba* flower were prepared by maceration technique and subjected to preliminary phytochemical tests. The *in-vitro* anti-microbial activity of all extracts was assessed by well diffusion method and antioxidant activity was assessed by UV-VIS spectrophotometry method. Methanolic extract showed significant (p<0.001) response when compared with standard, Ofloxicin and Amphotericin B (10 µg/ml). The study suggests that the extracts possess enough potential to reduce microbial infection and reduced the free radical activity by *in-vitro* and directs the importance of further research and development of novel antimicrobial and antioxidant agents.

Keywords: *Bombax cieba,* antioxidants activity, antimicrobial activity, UV-VIS spectrophotometer etc.

Introduction

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin [1]. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years [2]. According to World Health Organization (WHO) traditional medicines are relied upon by 65–80% of the World's population for their primary health care needs. Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine [3].

Throughout the world plants are used to treat various infectious diseases. They provide natural products that are used against infectious diseases. Plant-derived materials or products with therapeutic properties are known as herbal medicines; they may contain processed or raw ingredients from one or more plants that are beneficial for human health. Medicinal plants are important with respect to new drug and pharmacological research development. They are widely used and accepted as home remedies and raw materials for the pharmaceutical industry. Indigenous knowledge of plants and animals that are used to maintain health is known as Ethnopharmacology. The use of plants as medicines dates back to ancient times. Chinese physicians used Ephedra tea for asthma, hay fever and colds in 3,000 BC [4].

Recently, the use of medicinal plants increased substantially [5]. People use herbs to treat different diseases because they are cheap and effective, but doctors are often reluctant to prescribe them because of knowledge deficiency, real concerns about product safety, concerns about liability, and the presence of pathogens and compounds that are injurious [6,7]. Medicinal plants are very important for the cure of different microbial infections but heavy metals adversely affect bacterial viability [8] and activity [9]. Experiments on the use of plant compounds against microbes were first documented in the late 19th century [10]. Natural products perform various functions and many have interesting and useful biological activities [11]. Researchers are turning their attention to natural products to develop better anticancer, antiviral and antibacterial drugs [12,13,14]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [15,16,17].

Normal physiological processes involve utilization of oxygen in which approximately 5% of the oxygen gets reduced univalent to oxygen-derived free radicals. These radicals, known as reactive oxygen species (ROS), exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits

per second. When generation of ROS overtakes the antioxidant defence of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders such as glycated protein oxidation in diabetes mellitus, low-density lipoprotein oxidation in atherosclerosis, red blood cell haemolysis in glucose- 6- phosphate dehydrogenase deficiency, etc. These reactive species are capable of reversibly or irreversibly damaging compounds of all biochemical classes including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules [18].

Natural products have been used to prevent such types of damage since long. Many plants contain substantial amounts of antioxidants like vitamin C and E, carotenoids, flavonoids, tannins, etc. That can be used to scavenge the excess free radicals from human body. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanism can prevent and in some cases, help in the treatment of some oxidative related disorders [19].

In the present study the different extract of *Bombax cieba* of flower was selected for assess their antioxidant and antimicrobial properties. These plants are a common household remedy against a variety of gastrointestinal disorders, e.g. indigestion, flatulence, colin pain etc. [20].

Method and Material:
All the chemicals and standard antibiotics were purchased from Hi-Media, Mumbai, India; and al the solvents used was of analytical grade. Precoated silica gel 60 F254 TLC plates and standard phytoconstituents were purchased from Merck, Germany and Sigma Chemicals, USA, respectively.

Collection and Authentication of Plant:
Flower *Bombax cieba* (Bombacaceae), was collected from local area of Bharat scout bhawan, Bhopal. And seeds were obtained from local market from Bhopal. The authentications were done by Dr. Zia-Ul-hassan, Botanist at Govt. Safia College, Bhopal and herbarium was submitted to the department of botany, Govt. Safia College Bhopal. Specimen voucher no. is 426/BOT/SAFIA/17 and reference no is 444

Bacterial cultures:
Reference bacterial strains viz. *Proteus* (MTCC 2309), *B. Subtilis* (MTCC 1187), *Actinomyces* (MTCC 7802), *B. Cereus* (MTCC 1305), *A. Niger* (MTCC 0872), *C. krusie* (MTCC 9215) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. These were maintained on nutrient agar slants. All the isolates were sub cultured regularly and stored at 4°C as well as at - 80°C by making their suspension in 10% glycerol.

Preparation of plant extract:
Flower of *Bombax cieba*, was dried in shade and powdered. Maceration with different solvents viz. Petroleum ether, chloroform and methanol according to their polarity for 48 to 72 hours at room temperature and this procedure was repeated twice. The extracts were concentrated on a rotary evaporator and then dried with a spray dryer. The yield of the different extracts was 1.23%, 2.34%, and 3.2.4% [21,22].

Phytochemical investigation:
The Phytochemical screening of the plant extract was carried out by following methods [23,24,25] to detect the presence or absence of certain bioactive compounds.

Determination of Antimicrobial activity by Agar well diffusion method:
Sensitivity of different bacterial and fungal strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay [26]. The plates containing Mueller- Hinton/Nutrient agar were spread with 0.2 ml of the inoculum. Wells (8 mm diameter) were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml of the extract. The plates inoculated with different bacteria were incubated at 37°C up to 48 h and diameter of any resultant zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in duplicate and repeated thrice. The bacteria with a clear zone of inhibition of more than 12 mm were considered to be sensitive. The antimicrobial activity of different plant extracts was compared with commonly employed antibiotics viz. Ofloxicin (10 µg/ml) foe bacterial strains, and for fungi Amphotericin B (10 µg/ml).

Minimum inhibitory concentration (MIC):

Minimum inhibitory concentration of the effective extracts was worked out by broth dilution method [27]. The MIC is expressed in mg/ml. Broth dilution is a susceptibility testing technique in which serial dilutions (usually two-fold) of an antibacterial agent are made in a liquid medium that is inoculated with a standardised number of organisms and incubated for a prescribed period. Nutrient broth containing varying concentrations (10–100 mg/ml) of different extracts were prepared and inoculated with 0.1 ml of the inoculums. The test tubes were incubated at 37°C for 24 h and the lowest concentration of the extract causing complete inhibition of the bacterial growth was taken as MIC. The results were compared with that of control using sterilized distilled water/acetone. The experiment was performed in duplicate and repeated three times.

DPPH radical scavenging activity

15 mg of DPPH was dissolved in 10 ml of methanol. 75µl of this solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75µl of DPPH was added to a mixture of methanol and 50 µl of extract. The final volume was adjusted to 3 ml. Decrease in absorbance of the DPPH was measured 517 nm [28].

Reducing power assay

Prepare different concentration of test sample. Add 0.5 ml of different concentrations of sample with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V). Reaction mixture was incubated at 50° C for 20 min. After cooling, add 1.5 ml of trichloroacetic acid solution (10% W/V) to terminate the reaction. Add 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 770 nm. Plot a curve of absorbance versus concentration. Increased absorbance of the reaction mixture indicates increase in reducing power [29].

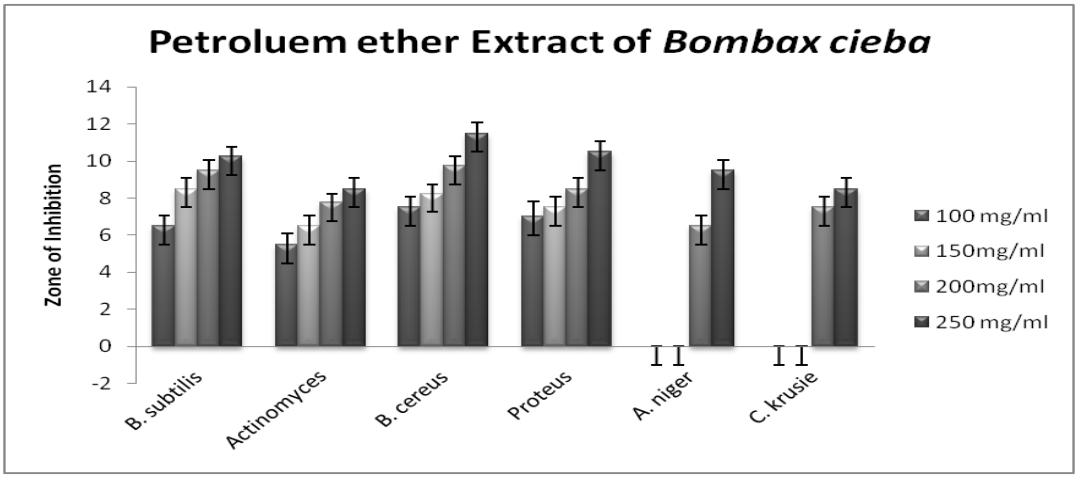
Results:

Table 1: Phytochemical investigation of *Bombax cieba* flower Extract

S. No.	Test	Petroleum ether	Chloroform	Methanol
1.	Carbohydrate	+	+	+
2.	Protein	-	-	+
3.	Alkaloids	-	+	+
4.	Flavonoids	-	+	+
5.	Glycosides	+	+	+
6.	Tannins & Phenolics	-	+	+
7.	Saponins	-	-	-
8.	Terpenoids & steroids	-	-	-

Table 2: Well Diffusion Assay of *Bombax cieba* flower Petroleum ether Extract

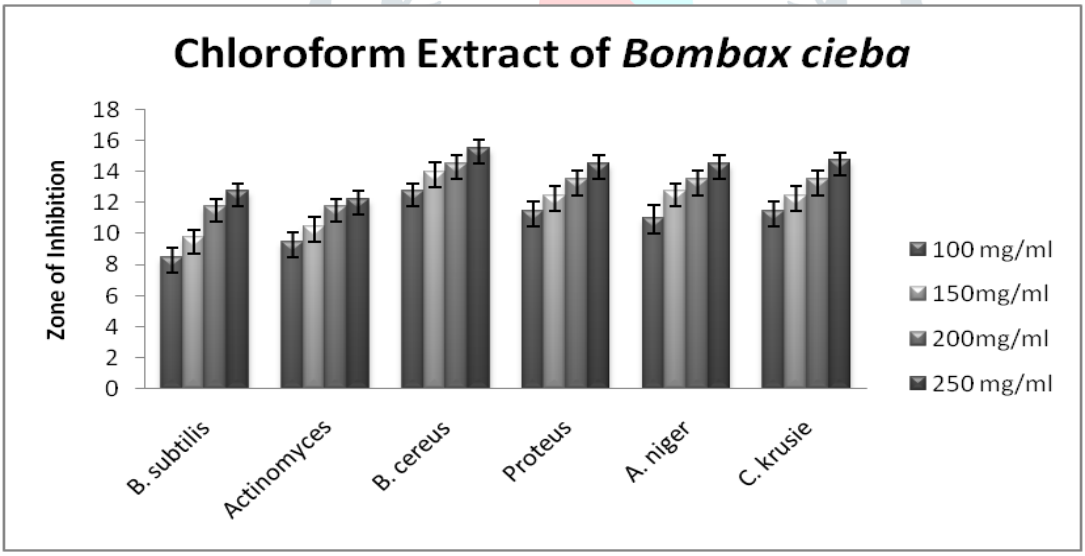
S. No.	Organisms	Petroleum ether extract of *Bombax cieba*			
		100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml
1.	*B. subtilis*	6.50±0.577	8.50±0.577	9.50±0.577	10.25±0.500
2.	*Actinomyces*	5.50±0.577	6.50±0.577	7.75±0.500	8.50±0.577
3.	*B. cereus*	7.50±0.577	8.25±0.500	9.75±0.500	11.50±0.577
4.	*Proteus*	7.00±0.816	7.50±0.577	8.50±0.577	10.50±0.577
5.	*A. niger*	0.00±0.000	0.00±0.000	6.50±0.577	9.50±0.577
6.	*C. krusie*	0.00±0.000	0.00±0.000	7.50±0.577	8.50±0.577



Graph 1: Well Diffusion Assay of *Bombax cieba* flower Petroleum ether Extract

Table 3: Well Diffusion assay of *Bombax cieba* flower Chloroform Extract

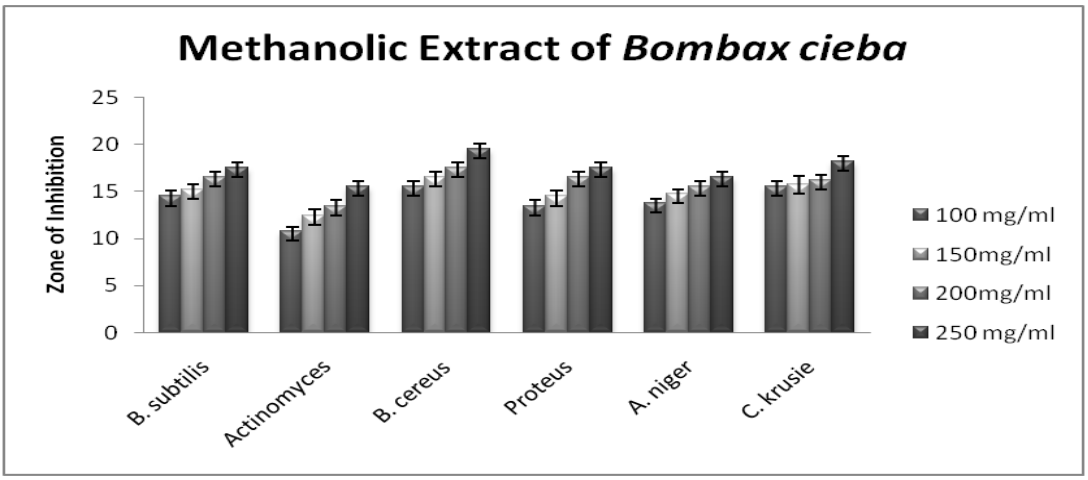
S. no.	Organisms	Chloroform extract of *Bombax cieba*			
		100 mg/ml	**150 mg/ml**	**200 mg/ml**	**250 mg/ml**
1.	***B. subtilis***	8.50±0.577	9.75±0.500	11.75±0.500	12.75±0.500
2.	***Actinomyces***	9.50±0.577	10.50±0.577	11.75±0.500	12.25±0.500
3.	***B. cereus***	12.75±0.500	14.00±0.577	14.50±0.577	15.50±0.577
4.	***Proteus***	11.50±0.577	12.50±0.577	13.50±0.577	14.50v0.577
5.	***A. niger***	11.00±0.816	12.75±0.500	13.50±0.577	14.50±0.577
6.	***C. krusie***	11.500.577	12.50±0.577	13.50±0.577	14.75±0.500



Graph 2: Well Diffusion assay of *Bombax cieba* flower Chloroform Extract

Table 4: Well Diffusion assay of *Bombax cieba* flower methanolic Extract

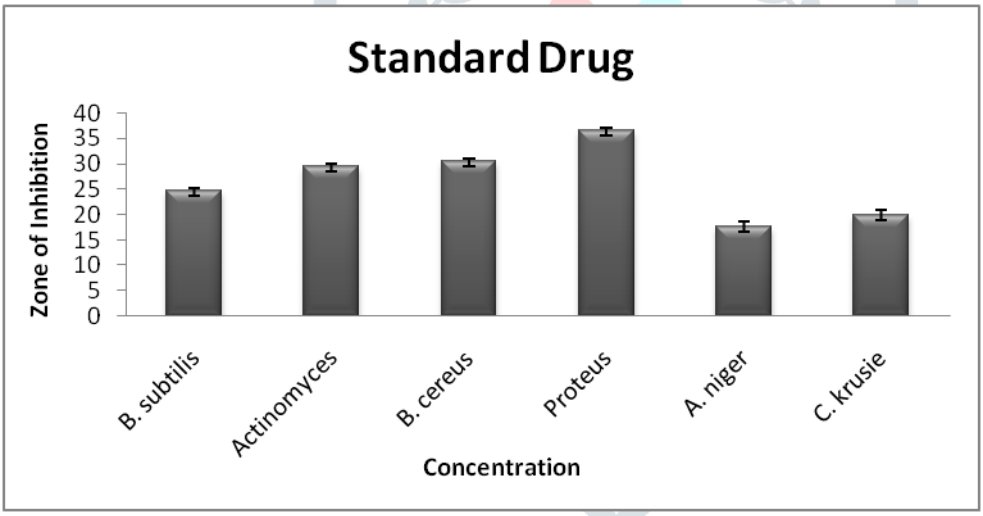
S. no.	Organisms	Methanolic extract of *Bombax cieba*			
		100 mg/ml	**150 mg/ml**	**200 mg/ml**	**250 mg/ml**
1.	***B. subtilis***	14.50±0.577	15.25±0.500	16.50±0.577	17.50±0.577
2.	***Actinomyces***	10.75±0.500	12.50±0.577	13.50±0.577	15.50±0.577
3.	***B. cereus***	15.50±0.577	16.50±0.577	17.50±0.577	19.50±0.577
4.	***Proteus***	13.50±0.577	14.50±0.577	16.50±0.577	17.50±0.577
5.	***A. niger***	13.75±0.500	14.75±0.500	15.50±0.577	16.50±0.577
6.	***C. krusie***	15.50±-.577	15.75±0.957	16.25±0.500	18.25±0.500



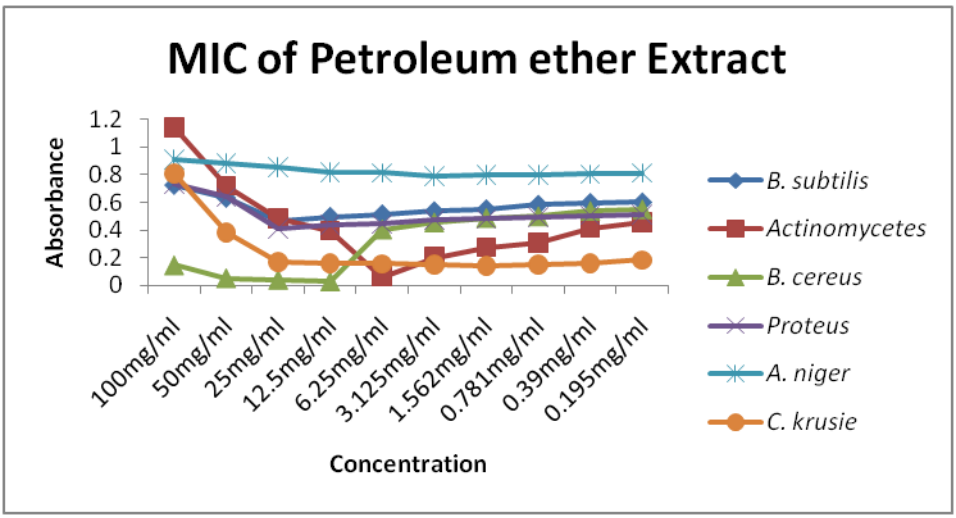
Graph 3: Well Diffusion assay of *Bombax cieba* flower methanolic Extract

Table 5: Well diffusion assay of Standard Drugs

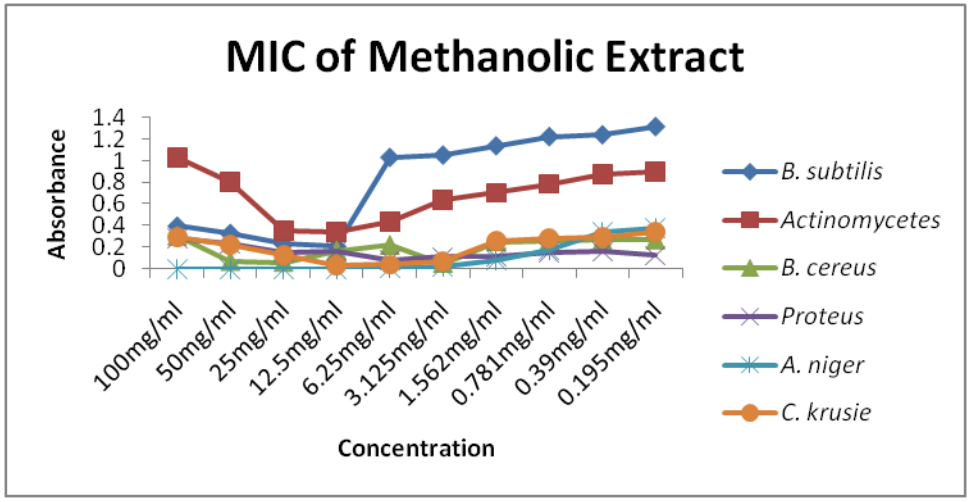
S. no.	Organisms	Zone of Inhibition	Standard drugs
1.	***B. subtilis***	24.75±0.500	Ofloxicin (10µg/ml)
2.	***Actinomyces***	29.50±0.577	
3.	***B. cereus***	30.50±0.577	
4.	***Proteus***	36.50±0.577	
5.	***A. niger***	17.75±0.957	Amphotericin B(10µg/ml)
6.	***C. krusie***	20.00±0.816	



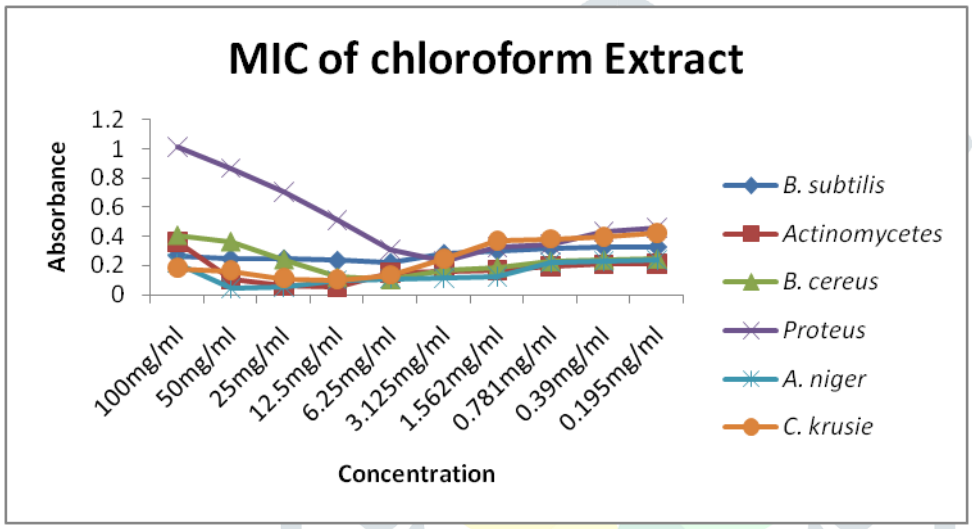
Graph 4: Well diffusion assay of Standard Drugs (ofloxicin for bacteria and Amphotericin B for fungi)



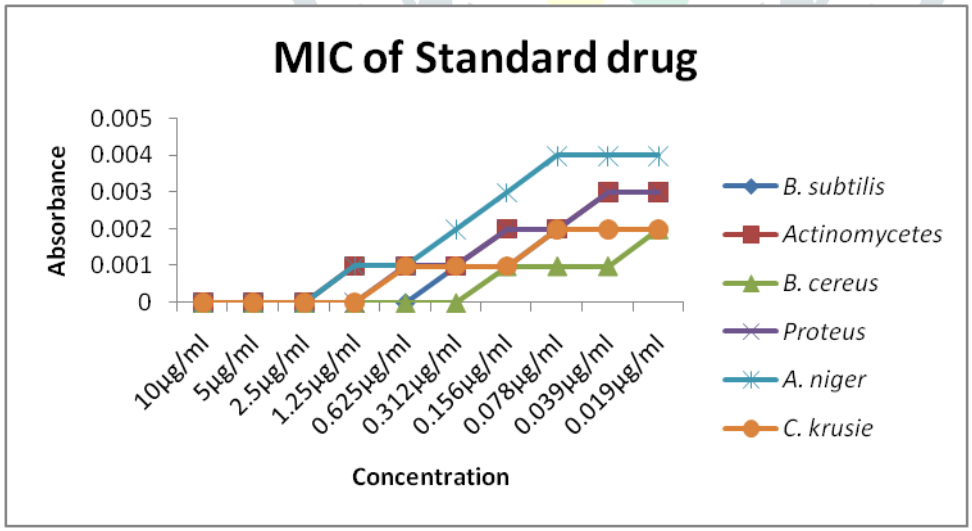
Graph 5: MIC of Petroleum ether extract of *Bombax cieba* in different concentrations



Graph 6: MIC of Methanolic extract of *Bombax cieba* in different concentrations



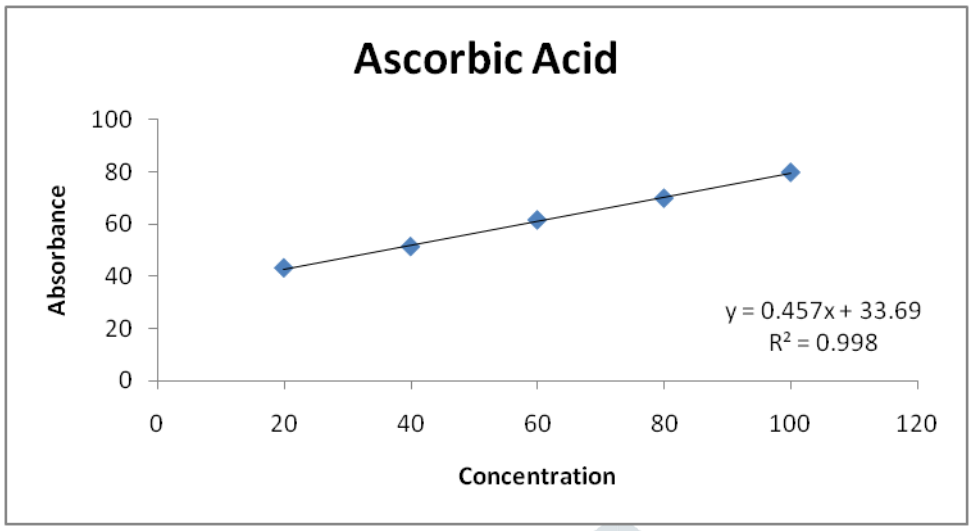
Graph 7: MIC of Chloroform extract of *Bombax cieba* in different concentrations



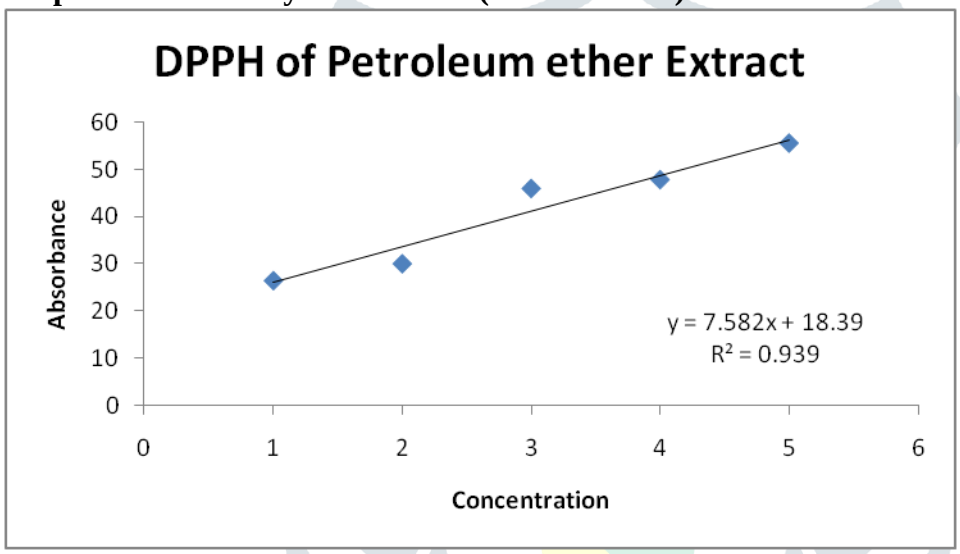
Graph 8: MIC of Standard drug in different concentrations

Table 6: DPPH assay of Standard (Ascorbic acid)

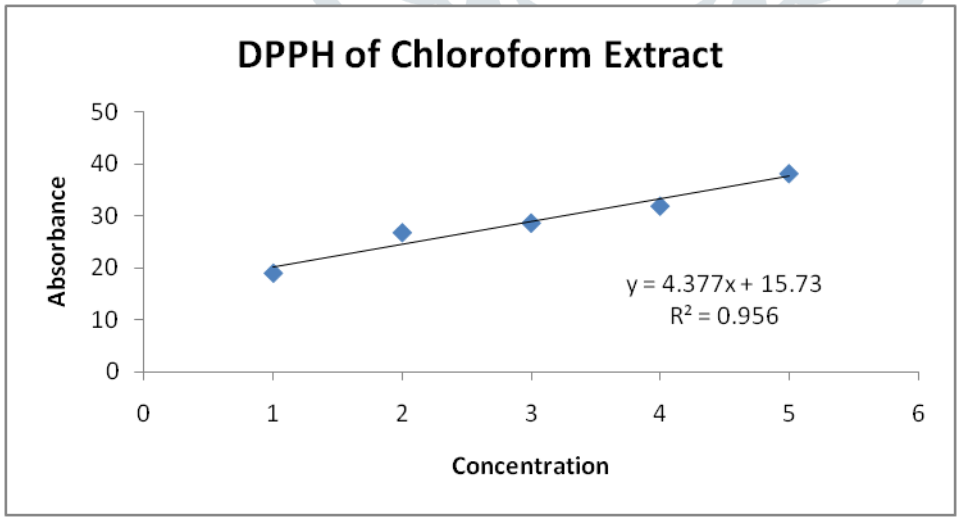
S. No.	Concentration (µg/ml)	Ascorbic Acid	Petroleum Ether	Chloroform	Methanol
1.	20	0.397	0.514	0.567	0.453
2.	40	0.340	0.489	0.512	0.423
3.	60	0.269	0.378	0.499	0.389
4.	80	0.211	0.365	0.476	0.365
5.	100	0.142	0.311	0.432	0.321



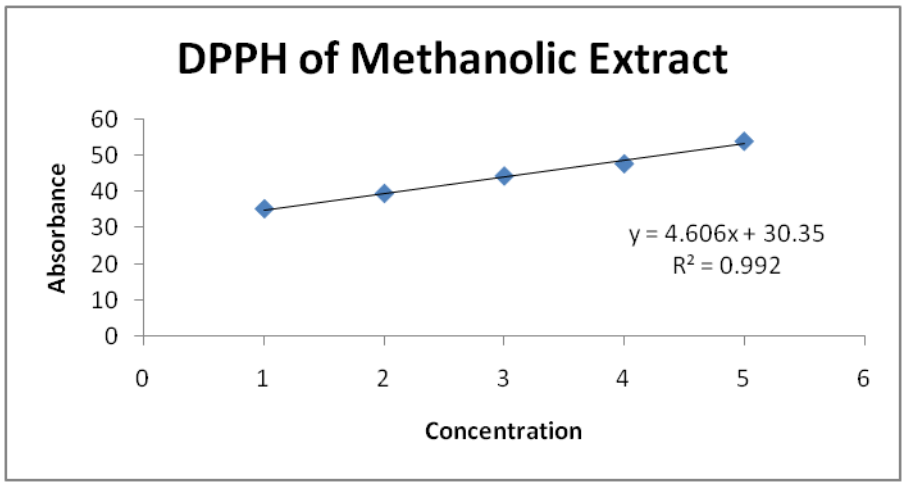
Graph 9: DPPH assay of Standard (Ascorbic acid)



Graph 10: DPPH assay of Petroleum ether extract of *Bombax cieba*



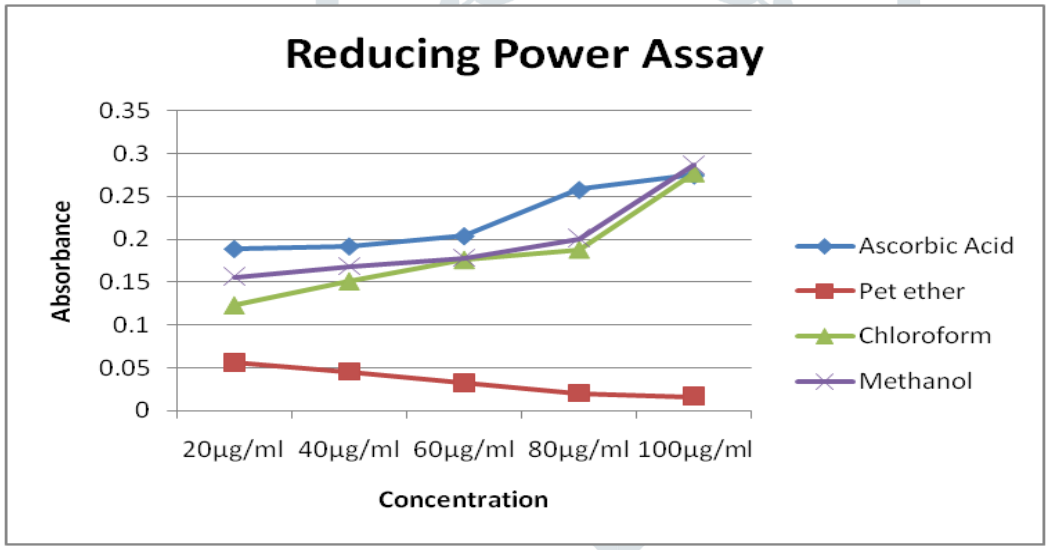
Graph 11: DPPH assay of Chloroform extract of *Bombax cieba*



Graph 12: DPPH assay of Methanolic extract of *Bombax cieba*

Table 7: Reducing Power assay of Standard (Ascorbic acid) of *Bombax cieba* flower Extract

S. No.	Concentration (µg/ml)	Ascorbic acid	Petroleum ether	Chloroform	Methanol
1.	20	0.189	0.056	0.123	0.156
2.	40	0.192	0.045	0.151	0.168
3.	60	0.204	0.032	0.176	0.178
4.	80	0.258	0.020	0.188	0.201
5.	100	0.275	0.016	0.277	0.287



Graph 13: Reducing power assay of different extracts of *Bombax cieba*

Discussion:

In recent years the use of herbs in traditional medicine has gained attention as they are being proven as the promising sources of various bioactive molecules. Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins and flavonoids in both the species [30]. Phenolics, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier [31]. Hence, these may be responsible for the observed activity in both these species.

B. cieba flowers have been shown to contain the β-Dglucoside of β-sitosterol, free β-sitosterol, hentriacontane, hentriacontanol, traces of an essential oil, kaempferol, and quercetin [32].

In the present experiment different extract were used for antimicrobial activity against various microorganisms. As the results shows Methanolic extract have a strong antimicrobial activity for all organisms as comparative to chloroform and petroleum ether extract. Whereas the *A. niger* and *C. krusie* has not show any zone of inhibition in petroleum extract at 150 mg/ml concentration (Table 2), therefore they has shown good zone of inhibition in the Methanolic extract at 100 mg/ml concentration (Table 4).

Methanolic extract also shows the good significance nearby standard drug (ofloxicin and Amphotericin-B) at the concentration of 10µg/ml for all organisms (Table 5).

The minimum inhibition concentration assay shows the significance with standard drug. Where the methanolic extract has the MIC value at 12.5 to 6.25 mg/ml, and standard drugs have the MIC value at 0.312 µg/ml.

Several concentrations ranging from 20 - 100 µg/ml of the petroleum ether, chloroform and methanolic extracts of were compared for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner up to the given concentration in all the models. On a comparative basis, both the extracts showed almost near values (Table 6). DPPH is a stable free radical. The in vitro study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up [33]. From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles. Ortho – substituted phenolic compounds may exert pro-oxidant effects by interacting with iron. O – phenanthroline quantitatively forms complexes with ferric ion which get disrupted in the presence of chelating agents. The extracts interfered with the formation of ferrous – O – phenanthroline complex, thereby suggesting that the extract has metal chelating activity. There is the similarity in the results of reducing power assay it reduces the radicals, like ascorbic acid do.

Thus, our experiments explore the antioxidant and antimicrobial activity of *Bombax cieba* and the plant secondary metabolites could be regarded as efficient scavengers of reactive oxygen species offering the cell protective function.

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