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Comparative evaluation of In-vitro antioxidant and antimicrobial potential of different extracts of bark and leaves of Morus alba

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Abstract:

This study aims to identify phytochemicals, assess the antioxidant capacity and antimicrobial activity of plant such as *Morus alba* by utilising a bark and leaves. A recognised testing approach was used to determine the qualitative analysis of several phytochemical components. An 50% ethanolic extract of *Morus alba* bark-leaf mixture was subjected to an in vitro antioxidant activity study using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay, superoxide radical scavenging assay, and reducing power determination. Phenols and flavonoids were found by phytochemical investigations. The petroleum ether, chloroform, ethyl acetate extract and 50% ethanolic extract of different parts like bark and leaves of *Morus alba* were tested for activity against *Streptococcus mutans*, *Salmonella bongori* and *Escherichia coli* pathogens by using agar well diffusion assays.

The blend of *morus alba* bark-leaves had an IC50 value of 63.061µg/ml, for the DPPH method, which was comparable to ascorbic acid (IC50=57.594 µg/ml). The extract exhibited dose-dependent free radical scavenging capacity in the models that were studied. In the models that were examined, the extract showed dose-dependent free radical scavenging ability. In the reducing power determination, the IC50 value was found to be 88.487 µg/ml, which was comparable to ascorbic acid's IC50 value of 77.762 µg/ml. The maximum antimicrobial activity was shown by 50% Ethanolic extract fraction of bark and leaves. The zone of inhibition of bark extract 19±0.15, mm, 21±0.72 mm, 22±0.51 mm was observed against *S. mutans, S. bongori, Escherichia coli* respectively. In case of leaves extract, zone of inhibition of 11±0.24 mm, 24±0.36 mm, 18±0.18 mm was observed against *S. mutans, S. bongori, Escherichia coli* respectively. This research describes the phytochemical profile, antioxidant activity and antimicrobial activity of *morus alba*, which will be used for therapeutic applications.

Keywords: *Morus alba*, antioxidant activity, antimicrobial activity, phytochemical investigations

1. Introduction

It has long been known that a number of flavonoid groups exhibit antioxidant action against substances that are easily oxidised.[1] Medicinal herbs are the primary source of antioxidants, which are substances that stop various molecules' oxidation chain processes.[2] A significant portion of phenolic chemicals, which function as antioxidants, are found in herbal plants. Redox properties of antioxidant substances demonstrate their action by scavenging free radicals and breaking down peroxides.[3]

One or more unpaired electrons can be found in free radicals, which are extremely unstable chemical entities. There are

different free radicals are possible. For exampleFree radicals (ROS) and RNS, produced from nitrogen and oxygen, respectively, can occur.Reactive oxygen species that are most frequently encountered include reactive hydroxyl radicals (OH), peroxyl radicals (ROO), hydrogen peroxide (H_2O_2), and superoxide anion (O_2). Nitric oxide (NO), dinitrogen trioxide (N_2O_3), peroxy nitrite anion (ONOO), and nitrogen dioxide (NO_2) are the free radicals produced from nitrogen.[4, 5] Cells, dormant enzymes, DNA, cell membranes, and polysaccharides have all been shown to be destroyed by superoxide radicals.[6] Within the human body, endogenous metabolic activity and external substances combine to form free radicals. These oxidise biomolecules such nucleic acids, proteins, lipids, and DNA, which can lead to neurological diseases, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative ailments.[7, 8] Numerous illnesses, including diabetes, neurological diseases, and ageing, have also been linked to free radicals.[9, 10] Some plants have the ability toneutralise free radicals, according to earlier research. Ayurvedic remedies include a variety of extracts from medicinal plants that are known to contain certain physiologically active ingredients; these extracts are made in large quantities for export.The antioxidant activity of plant extracts, such as the fruit and bark of *Morus alba*, was assessed in the current study using a variety of in vitro test techniques, including DPPH scavenging activity, superoxide radical, suppression of microsomal lipid peroxidation, and reducing power.[11]

Due to the large variety of bioactive components they create, plants are an abundant source of different kinds of medications.[12, 13] They are important for both individual and community health and quality of life, and they have a big impact on both. A number of traditional medicinal plants are utilised as seasonings and food.[14, 15] To varying degrees, nearly every organism is shielded from the damaging effects of free radicals by antioxidant molecules such as glutathione, ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and superoxide dismutase. Prior and Cao suggest using dietary antioxidants or antioxidant supplementation.[16] Ward off the damaging effects of free radicals. As a way to stop free radicals from causing oxidative damage to the human body-especially to the brain-natural antioxidants are becoming more and more popular. Using conventional psycho neuropharmacology techniques, a number of medicinal plants have shown effective within the past twenty years.[17] Vegetables and fruits also include a variety of antioxidant chemicals, such as tocopherols, phenolics, carotenoids, and anthocyanins. Plants are capable of producing a wide variety of bioactive compounds. Fruits and vegetables include higher amounts of phytochemicals that may offer protection against damage from free radicals.[18] The plant known as *Morus alba* has been utilised in traditional medicine for a very long time because of its unique chemical makeup and medicinal properties. Chinese and Indian medicine use components of the mulberry plant, although not all of them.[19] Mulberry is known as "Toola, Tula" in Sanskrit and "Chinni, Tut, Tutri" in Hindi and Tamil.[20] The white mulberry, or *Morus alba* (family: Moraceae), is a plant that is native to Asia, Europe, North and South America, Africa, and India. It has been domesticated for thousands of years and is known by several names, including Tut in the local dialect. In sericulture, it is widely grown for its leaf yield.[21] According to reports, mulberries have numerous antioxidant flavonoid components and have the ability to scavenge free radicals. They are frequently employed as an alternative medicine and as part of the silkworm diet.[22] In both its raw and extracted forms, medicinal plants are used to treat a variety of microbial diseases. It is commonly recognized that plant products are the source of even the most manufactured medications. A significant portion of the world's healthcare system, in both industrialized and developing nations, is still beset by the issue of antibiotic resistance. A significant burden on health services and an issue that has not yet been addressed is the emergence of multidrug-resistant (MDR) bacteria in hospital and community settings.[23] Crude extracts from bark and leaves have antimicrobial properties against certain specific pathogenic bacteria that are locally accessible for the potential creation of novel medications for the prevention and treatment of infectious disorders brought on by bacterial pathogens. Comparing *Morus alba's* antioxidant and antimicrobial properties to those of other plants that have been used historically for a variety of purposes was the aim of the current study.

2. Material and Method

2.1 Material (Sample collection (Plant)

Between July and August, the leaves and bark of the *Morus alba* plants were gathered from the garden of CSA University, Kanpur, India. Dr. Naveen K. Ambasht, a Head and Associate Professor at the Botany Department, Christ Church College, Kanpur who specializes in plant taxonomy, recognized and verified the plants. To get rid of any unwanted material, the plant material was cleaned with water and then dried in the shade.

2.2 Chemicals and standards

Most of the reagents used in this work were provided by Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India). In this experiment, every chemical and solvent employed was of analytical quality.

2.3 Extraction by maceration process

During the maceration process, 200 g of a powdered mixture of *Morus alba* leaves and bark were extracted using a variety of solvents of choice, including petroleum ether, chloroform, ethyl acetate, and 50% ethanol.[24] To extract the mixture of powdered form of leaf and bark, 200 g of it was individually soaked in 100 mL of various solvents in a conical flask. The mixture was then allowed to stand at room temperature for three days before being filtered using Whatman No. 1 filter paper.[25] At temperatures greater than their boiling points, the extract evaporated. Finally, the % yield of the dried extracts was computed. Subsequently, the extracts were reduced in a rotary evaporator and stored for later use at 4°C in airtight containers.

2.4 Phytochemical screening of the extract

The first phytochemical screening of the crude extracts is thereafter conducted using the procedure's conventional techniques. The phytoconstituents that were qualitatively examined in the extracts of *Morus alba* bark and leaves included alkaloids, proteins, amino acids, glycosides, carbohydrates, phytosterols, saponins, tannins, vitamins, and flavonoids.[26, 27]

2.5 *In-vitro* antioxidant activity of 50% Ethanolic extract of *Morus alba* leaves-bark mixture using different method DPPH method

The activity of DPPH scavenging was measured with a spectrophotometer.[28] The standard approach was utilised to assess the DPPH (1,1-Diphenyl-2-Picrylhydrozyl) free radical scavenging activity of *Morus alba* in 50% ethanoilc extract.[29] 1.5 ml of a 0.004% methanol solution of DPPH was mixed with the different bark-leaf extract concentrations. The concentrations were then allowed to incubate for 30 minutes at room temperature. At 517 nm, absorbance was measured in relation to a blank. The conventional control was ascorbic acid.[30] To get an initial absorbance of 1.5 mL in 1.5 mL methanol, the stock solution (6 mg in 100 mL methanol) was prepared. When sample extract was present for 15 minutes at different concentrations (10–100g/ml), there was a drop in absorbance. Using methanol, the final volume was adjusted to 3 ml after 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added to a series of volumetric flasks.

The same procedures were followed for the collection and processing of three test samples. Ultimately, the mean was computed. The absorbance of DPPH at various doses showed a final decline at 517 nm after 15 minutes.

Using the following formula, the absorbance measurements were utilised to determine the proportion of antioxidant activity (% inhibition):[31]

$$\textbf{Calculation of \% inhibition} = \frac{\textbf{Control absorbance}(\text{A0}) - \textbf{Test absorbance}\ (\text{A1})}{\textbf{Control absorbance}(\text{A0})} X100$$

2.6 Superoxide radical scavenging assay

The extracts' ability to scavenge superoxide radicals (O_2) was determined using the NBT (Nitro tetrazolium blue) reagent method. The reaction mixture contained varying amounts of the extract together with PMS (phenazinemethosulfate) (0.1 mmol/L), NADH (1 mmol/L), and NBT (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4). The extracts' capacity to scavenge superoxide radicals was assessed with a maximum wavelength of 560 nm, the nitrite ion produced a colour

when exposed to EDTA.[32] The approach relies on the production of O_2 by the autooxidation of hydroxylamine hydrochloride in the presence of NBT. The extracts (1 mL, 10-100 µg/mL) test samples were mixed with NBT (0.4 mL, 24 mM), EDTA (0.2 mL, 0.1 mM), and sodium carbonate (1 mL, 50 mM) solutions. After five minutes of room temperature incubation, the mixture's colour was measured at 560 nm in comparison to a blank. The following formula was used to determine the scavenging effect.[33]

$$\text{Calculation of \% Reduction} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \text{X100}$$

2.7 Reducing power determination

The ability of extracts to reduce ferric iron was examined using the potassium ferricyanide-ferric chloride technique. 0.2 mL of each extract at different concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1 percent) were combined and incubated at 50°C for 20 minutes in order to reduce ferric iron to ferrocyanide.

Centrifuging for ten minutes at 1000 rpm and adding 2.5 mL of 10% (w/v) trichloroacetic acid stopped the process. The absorbance was then measured at 700 nm using 2.5 mL of the upper layer, 2.5 mL of distilled water, and 0.5 mL of 0.1 percent $FeCl_3$.

2.8 Antimicrobial Analysis

The in vitro antimicrobial efficacy of petroleum ether, chloroform, ethyl acetate, and 50% ethanolic extracts of *Morus alba* leaves and bark was examined against Gram-positive (*Streptococcus mutans*) and Gram-negative (*Salmonella bongori and Escherichia coli*) bacteria. The antibacterial activity of the extracts of *Morus alba* L. was assessed using the well diffusion method. The standard formulation used IP-grade ofloxacin and ciprofloxacin. For 24 hours, *S. Mutans, S. bongori* and *Escherichia coli* were cultivated to investigate their antibacterial efficacy. For each extracted phytochemical, three concentrations—10, 20, and 40 mg/ml were used in antibiogram investigations.[34]

2.8.1 Plate Preparation

Pre-sterilized petri dishes with a 95 mm diameter were filled with 30 ml of pre-autoclaved Mueller-Hinton agar (MHA). Room temperature was used to allow these petri plates to harden.[35]

2.8.2 Well Diffusion Method

Following the plates' solidification, a freshly made microbial broth culture suspension (about 0.2
ml) was individually sterilized and used an L-shaped glass spreader to distribute the suspension over the Mueller-Hinton agar (MHA) media in an aseptic setting with laminar air flow. Then, using an 8 mm diameter borer, wells were created in each plate. Approximately 0.2 ml of the extracts of fruit peel and leaves were each put into these wells separately.

In order to completely inhibit the growth of additional microorganisms in a circular area or zone surrounding the hole containing leaf and fruit peel extract, this method relies on the diffusion of extracts from the hole through the solidified agar layer of the petri dish. In the incubator, petri plates were incubated for 24 hours at 37^0C. Following incubation, the well or holes' diameter of the clear zone of inhibition was measured in millimetres and compared to a reference medication. (Table-7, 8).[36]

3. Results and Discussion

The percentage extraction yield varied from 1.97% to 17.82%, as indicated in Table 1. The highest percentage yields from leaves and stem bark were 17.21% and 17.82%, respectively, for the 50% ethanolic extracts. It was found that the yield of extract made using 50% Ethanolic solvents from a blend of *Morus alba* leaves and bark was 11.68 percent.

% Extractive yield

$$\% \text{ Extractive yield} = \frac{\text{Weight of Extract}}{\text{Weight of Crude Drug}} X100$$

Table 1. Extractive values of leaves and bark of *Morus alba*

S.No.	Extracts	%Yield (W/W)	
		Leave	Bark
1	Petroleum Ether	1.97	2.14
2	Chloroform	4.29	4.76
3	Ethyl Acetate	5.75	5.91
4	50% Ethanol	17.21	17.82

3.1 Phytochemical screening of the extract

The outcomes of a qualitative phytochemical analysis of the raw powder leaves and bark of *Morus alba* are presented in Table 2. 50% Ethanolic extract contained flavonoids, alkaloids, saponins, phenolics, carbohydrates, and tannins.

Table 2. Phytochemical screening of the extract:

S. No.	Constituents	Chloroform extract		Ethyl acetate extract		50% Ethanolic extract		Petroleum ether	
		leaves	bark	leaves	bark	leaves	bark	leaves	bark
1.	**Alkaloid**	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
2	**Glycosides:** Legal'sTest:	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
3	**Flavonoids:** LeadacetateTest: Alkaline test:	 -ve +ve	 +ve +ve	 +ve +ve	 +ve +ve	 +ve +ve	 -ve -ve	 +ve +ve	 -ve +ve
4	**Diterpenes** Copper acetate Test:	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
5	**Phenol:** Ferric Chloride Test:	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
6	**Proteins:** Xanthoproteic Test:	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
7	**Carbohydrate** Fehling'sTest:	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
8	**Saponins** Froth Test:	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve
9	**Tannins:** Gelatintest:	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
10	**Triterpenoid**	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve

3.2 DPPH assay of *Morus alba* leaves and bark extracts:

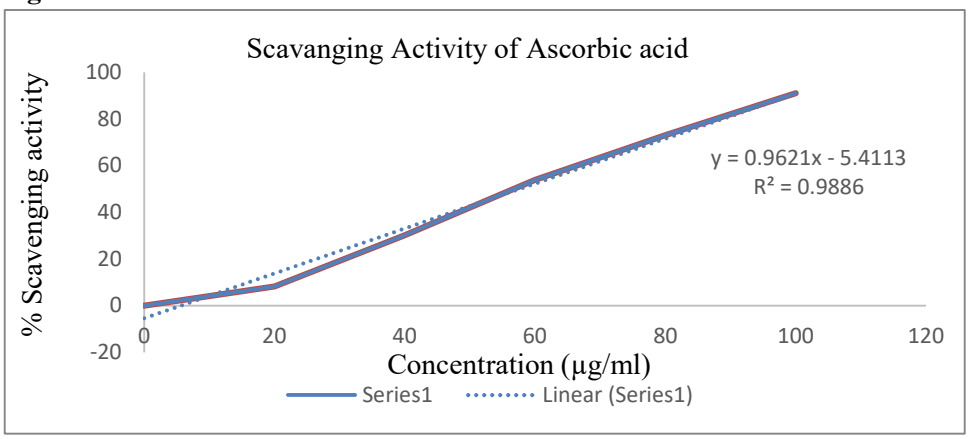
The DPPH radical scavenging assay was used to assess the extracts' ability to donate hydrogen. The DPPH radical scavenging activity of the leaves and bark mixture of *Morus alba* leaves-bark extract was measured using a variety of techniques to find the inhibitory concentration fifty percent (IC50) value. The extract shown dose-dependent capabilities

for scavenging free radicals in the models under investigation. The IC50 value of the leaves and bark mixture of *Morus alba* extract for the DPPH technique was 63.061µg/ml, respectively. This was comparable to the ascorbic acid (IC50=57.594 µg/ml) table 3 and figure 1, 2.

Table 3. % Inhibition of ascorbic acid and *Morus alba* leaves and bark mixture extract using DPPH method

Concentration (µg/ml)	Absorbance of Ascorbic Acid	Scavenging Activity of Ascorbic Acid	Absorbance of 50% ethanolic extract	Scavenging activity of 50% ethanolic extract
0	0.682±0.04	0	0.682±0.04	0
20	0.626±0.07	8.211±0.01	0.639±0.002	6.304±0.002
40	0.477±0.02	30.058±0.02	0.521±0.01	23.607±0.001
60	0.315±0.09	53.812±0.015	0.359±0.007	47.360±0.009
80	0.184±0.02	73.02±0.0.06	0.225±0.005	67.008±0.003
100	0.061±0.00	91.055±0.003	0.102±0.009	85.043±0.01
Control	0.682±0.04	0.682±0.04	0.682±0.04	0.682±0.04

Figure 1. % Inhibition of ascorbic acid and mixture of *Morus alba* leaves and bark extract using DPPH method



Calculation for scavenging activity:

IC_{50} of Ascorbic Acid

y = 0.9621x - 5.4113

Where Y= % Scavenging activity

X= Concentration (µg/ml)

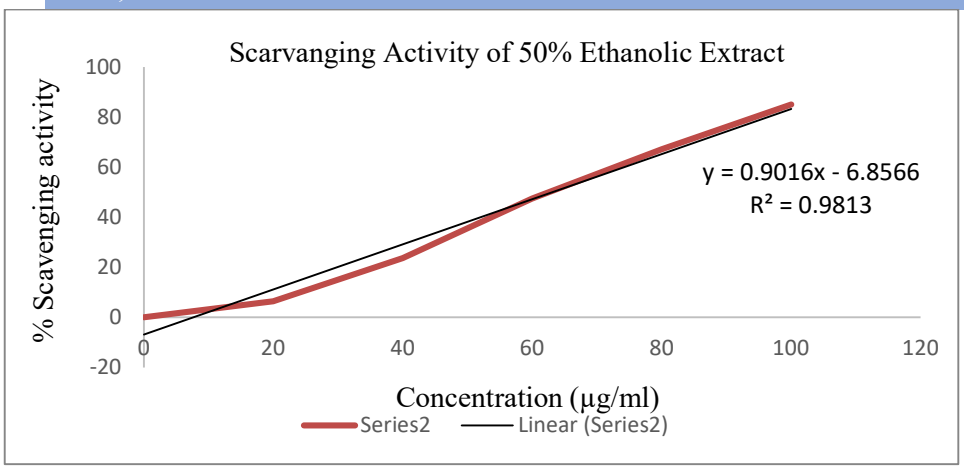
So, For 50% scavenging activity of ascorbic acid

50 = 0.9621x - 5.4113

$$X = \frac{50 + 5.4113}{0.9621}$$

IC_{50}= 57.594 ± 0.11µg/ml

Figure 2. Scarvanging Activity of 50% Ethanolic Extract



Calculation for Scavenging activity of 50% Ethanolic Extract:

$IC_{50}$of 50% Ethanolic Extract

y = 0.9016x - 6.8566

Where Y= % Scavenging activity

X= Concentration (µg/ml)

So, For 50% scavenging activity of 50% Ethanolic Extract

50 = 0.9016x - 6.8566

$$X = \frac{50 + 6.8566}{0.9016}$$

IC_{50}= 63.061 ± 0.08 µg/ml

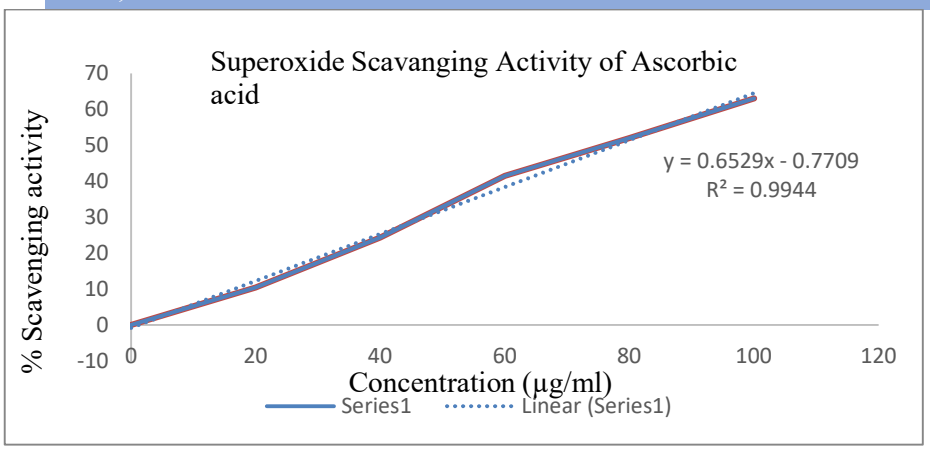
3.3 Superoxide Radical Scavenging:

The Superoxide Radical Scavenging Experiment's IC50 result was 88.487 µg/ml which compares favorably to ascorbic acid (IC50= 77.762 µg/ml) in Figure 3 and Table 4. The ascorbic acid (IC50= 77.762 µg/ml) table 4 and figure 3 were comparable to the IC50 value of 88.487 µg/ml table 4 and figure 4 found in the reducing power determination. This research describes the antioxidant activity and phytochemical profile of *Morus alba* leaves-bark extract, which will be used for therapeutic applications.

Table 4. Superoxide radical scavenging activity of 50% Ethanolic extract with reference to ascorbic acid

Concentration (µg/ml)	Absorbance of Ascorbic Acid	Superoxide Activity of Ascorbic Acid	Absorbance of 50% ethanolic extract	Superoxide activity of 50% ethanolic extract
0	0.593±0.007	0	0.593±0.007	0
20	0.531±0.013	10.455±0.031	0.531±0.005	10.455±0.031
40	0.449±0.017	24.283±0.030	0.489±0.003	17.537±0.094
60	0.347±0.009	41.483±0.097	0.391±0.012	34.064±0.008
80	0.285±0.004	51.939±0.029	0.338±0.006	43.001±0.068
100	0.219±0.001	63.069±0.013	0.241±0.005	59.359±0.019
Control	0.593±0.007	0.593±0.007	0.593±0.007	0.593±0.007

Figure 3. Superoxide Scavanging Activity of Ascorbic acid



Calculation for Superoxide Scavenging Activity of Ascorbic Acid:

IC_{50} of Ascorbic Acid

y = 0.6529x - 0.7709

Where Y= % Scavenging activity

X= Concentration (µg/ml)

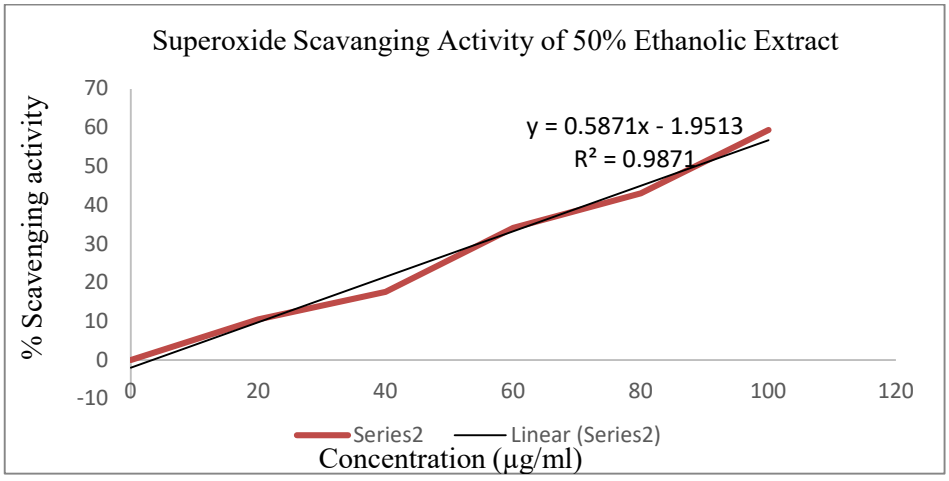
So, for 50% Superoxide Scavenging of ascorbic acid

50 = 0.6529x - 0.7709

$$X = \frac{50 + 0.7709}{0.6529}$$

IC_{50}= 77.762 ± 0.13 µg/ml

Figure 4. Superoxide Scavanging Activity of 50% Ethanolic Extract



Calculation for Superoxide Scavenging Activity of 50% Ethanolic Extract:

IC_{50} of 50% Ethanolic Extract

y = 0.5871x - 1.9513

Where Y= % Scavenging activity

X= Concentration (µg/ml)

So, For 50% Superoxide Scavenging of 50% Ethanolic Extract

50 = 0.5871x - 1.9513

$$X = \frac{50 + 1.9513}{0.5871}$$

IC_{50}= 88.487 ± 0.09 µg/ml

Table 5. Reducing power determination of ascorbic acid and 50% ethanolic extract of *Morus alba* leaves-bark extract

Concentration	Absorbance of Ascorbic Acid	Superoxide Activity of Ascorbic Acid	Absorbance of 50% ethanolic extract	Superoxide activity of 50% ethanolic extract
0	0.381±0.008	0	0.381±0.008	0
20	0.319±0.002	16.272±0.096	0.294±0.007	22.834±0.064
40	0.227±0.005	40.419±0.094	0.217±0.002	43.044±0.061
60	0.175±0.014	54.068±0.024	0.126±0.006	66.929±0.013
80	0.106±0.003	72.178±0.047	0.042±0.005	88.976±0.037
100	0.018±0.007	95.275±0.059	0.012±0.007	96.850±0.039
Control	0.381±0.008	0.381±0.008	0.381±0.008	0.381±0.008

Figure 5. Reducing power activity of ascorbic acid

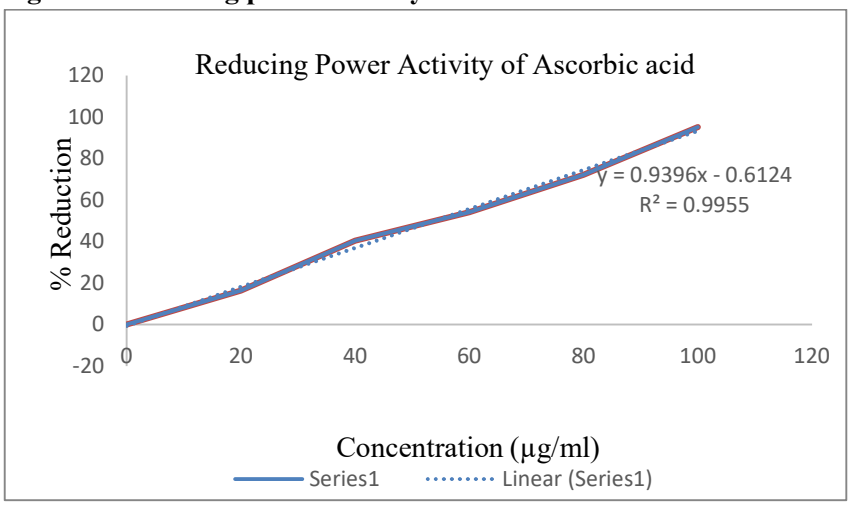


Figure 6. Reducing Power Activity of 50% Ethanolic Extract

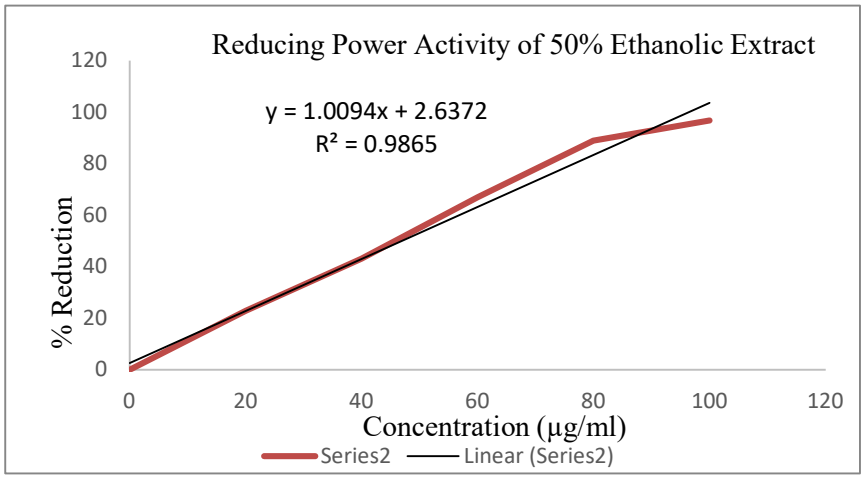


Table 6. Antimicrobial activity of different crude extracts of *Morus alba* leaves and bark extract against *Streptococcus mutans*, *Salmonella bongori* and *Escherichia coli*

Crude Extract	Concentration	Zone of inhibition (mm)					
		S. mutans		*S. bongori*		*E. coli*	
		Leaf	Bark	Leaf	Bark	Leaf	Bark
Petroleum ether	10 mg/ ml	8 ± 0.32	8 ± 0.13	6 ± 0.32	4 ± 0.11	6 ± 0.32	5 ± 0.23
	20 mg/ ml	10 ± 0.46	11 ± 0.44	9 ± 0.46	8 ± 0.32	9 ± 0.46	10 ± 0.28
	40 mg/ ml	13 ± 0.73	12 ± 0.54	14 ± 0.73	11 ± 0.51	10 ± 0.73	9 ± 0.62
Chloroform	10 mg/ ml	6 ± 0.12	7 ± 0.21	10 ± 0.41	9 ± 0.43	8 ± 0.10	7 ± 0.14
	20 mg/ ml	8 ± 0.31	7 ± 0.62	14 ± 0.62	9 ± 0.28	12 ± 0.47	11 ± 0.42
	40 mg/ ml	11 ± 0.42	10 ± 0.23	15 ± 0.24	12 ± 0.64	14 ± 0.51	14 ± 0.51
Ethyl acetate	10 mg/ ml	6 ± 0.12	7 ± 0.21	11 ± 0.46	10 ± 0.52	9 ± 0.20	8 ± 0.62
	20 mg/ ml	8 ± 0.31	7 ± 0.62	9 ± 0.21	11 ± 0.31	11 ± 0.47	11 ± 0.25
	40 mg/ ml	11 ± 0.42	10 ± 0.23	13 ± 0.31	15 ± 0.12	16 ± 0.32	16 ± 0.19
50% Ethanolic	10 mg/ ml	16 ± 0.34	12 ± 0.21	18 ± 0.11	17 ± 0.42	16 ± 0.42	14 ± 0.45
	20 mg/ ml	19 ± 0.43	15 ± 0.52	20 ± 0.62	18 ± 0.61	21 ± 0.72	19 ± 0.12
	40 mg/ ml	11 ± 0.24	19 ± 0.15	24 ± 0.36	21 ± 0.72	24 ± 0.18	22 ± 0.51
Ofloxacin	10 mg/ ml	18 ± 0.12	13 ± 0.21	20 ± 0.62	16 ± 0.82	21 ± 0.30	13 ± 0.61
	20 mg/ ml	22 ± 0.36	19 ± 0.52	25 ± 0.19	18 ± 0.61	24 ± 0.19	19 ± 0.45
	40 mg/ ml	28 ± 0.62	23 ± 0.38	28 ± 0.36	24 ± 0.10	26 ± 0.22	22 ± 0.27

* The values are in Mean ± SD, the average of three determinations

Gram-positive (*Streptococcus mutans*) and Gram-negative (*Salmonella bongori and Escherichia coli*) bacteria were among the microorganisms against which the in vitro antibacterial activity of petroleum ether, chloroform, ethyl acetate, and 50% ethanolic extracts of *Morus alba* leaves and bark was examined. The diameter of the clear zone in cultures in petriplates was used to quantitatively evaluate the crude extracts' antibacterial sensitivity and potency; the results are shown in Table 6. At 10, 20, and 40 mg/ml, the extracts of *Morus alba* exhibited modest antibacterial activity against every microorganism tested. Leaf extract was found to have a stronger antibacterial potential. The bioactive compounds (alkaloids, flavonoids, phenols, saponins, steroids, and tannins) found in the extracts of *Morus alba* plant parts in their leaves may be the reason why the extracts were able to stop the test bacteria from growing. The findings also demonstrated the high potential of *Morus alba* bark and leaf extracts as strong antibacterial agents. This correlation is supported by the bark extract's relatively weak antibacterial profile, which is suggested by its lower quantities of terpenoids and alkaloids, as shown by the quantitative study.

4. Conclusion

The investigation's findings suggest that the extract might effectively scavenge a variety of reactive oxygen species and free radicals in vitro. Its capacity to oxidize or undergo radical scavenging and produce a significant number of stable oxidized products may be the cause of this. The extracts' wide variety of activities suggests that antioxidant action is mediated by several pathways. The potential usefulness of the plants is demonstrated by the study's depiction of the extract's varied antioxidant effects. However, before these plants may be used as an antioxidant element in animal feeds or human health meals, their *in vivo* safety must be thoroughly examined in experimental rat models.

Petroleum ether, chloroform, ethyl acetate, and 50% ethanolic crude extracts from leaves and bark demonstrated the maximum effectiveness against the used bacteria, according to the current antimicrobial study of several crude extracts of *Morus alba*. Likewise, the maximum antioxidant activity was found in a 50% ethanolic crude extract of the leaves and bark. The presence of phytochemicals such alkaloids, flavonoids, phenols, saponins, steroids, and tannins is

necessary for the antioxidant and antibacterial properties of *Morus alba* crude extracts, according to phytochemical screening. Crude extracts from this plant may be a source of novel antioxidant and antibacterial compounds. To isolate and identify the active ingredients in the extracts that may have pharmacological use, more investigation is required.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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