



Investigation of Antioxidant and Antimicrobial Potential of Chloroform and Petroleum Ether Extracts of Selected Medicinal Plants of Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AHMZ and PPR designed the study, performed the statistical analysis, wrote the protocol, and managed the analysis of the study. Author MAMM wrote the first draft of the manuscript. Authors MSK and IJB managed the literature searches & revised the manuscript. Author TA revised the manuscript. Author MSR managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To investigate the antioxidant and antimicrobial potential of Chloroform and Petroleum ether extracts of *Manilkara zapota* (MZCE, MZPE), *Polyalthia longifolia* (PLCE, PLPE), *Abroma augusta* (AAPE, AAPE) *Ficus hispida* (FHCE, FHPE), *Vitex negundo* (VNCE, VNPE) plants.

Study Design: *In vitro* antioxidant and antimicrobial study.

Place and Duration of Study: Department of Pharmacy, School of Science & Engineering, Southeast University, Banani, Dhaka between June 2011 and March 2012.

Methodology: *In vitro* antioxidant activity was performed using DPPH radical scavenging, nitric oxide (NO) scavenging, reducing power, total antioxidant capacity, total

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phenol and total flavonoid content determination assays. The antimicrobial assay was performed by disc diffusion method using kanamycin and Nystatin as the standard.

Results: The most prominent antioxidant activity was observed with PLPE in DPPH radical scavenging test ($IC_{50} = 191.308 \pm 28.450 \mu\text{g/ml}$) as opposed to that of standard ascorbic acid ($IC_{50} = 43.129 \pm 1.181 \mu\text{g/ml}$). In total antioxidant capacity method, FHCE showed the highest activity ($837.558 \pm 110.835 \text{ mg ascorbic acid/g}$). The total phenolic and flavonoids content were determined by Folin–Ciocalteu Reagent and aluminum chloride colorimetric method respectively. The highest total phenols & total flavonoids content were found in VNPE ($180.434 \pm 142.19 \text{ mg Gallic acid/g}$ & $1265.255 \pm 165.593 \text{ mg quercetin/g}$, respectively). The ferric reducing capacity of the extracts was strong and dose dependent manner. PLPE displayed the highest antimicrobial actions against *Bacillus megaterium* (40 mm).

Conclusion: Comparison of different plant extracts used in the present study in various tested models showed wide variations in phenolic content and varying degrees of radical scavenging & reducing capacity. The obtained results indicate that investigated plants could be potential sources of natural antioxidants & antimicrobial agents and can be used for infectious diseases.

Keywords: Antioxidant; antimicrobial; chloroform; petroleum ether; medicinal plant.

1. INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds [1]. About 50% drug used in medicine and 25% of prescribed drugs in the world are of plant Origin[2,3]. In developing countries about 80% of people rely on traditional plant based medicines for their primary health care needs[2]. To treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders natural products and related drugs are used [4].

Undoubtedly Oxygen is an indispensable part of aerobic life. However, under certain circumstances, through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species, it can seriously affect our well being [5,6]. Hydroxyl radical ($\text{HO}\bullet$), the superoxide radical ($\text{O}_2\bullet^-$), the nitric oxide radical ($\text{NO}\bullet$) and the lipid peroxy radical ($\text{LOO}\bullet$) are the most frequently encountered free radicals, while H_2O_2 and singlet oxygen ($^1\text{O}_2$) are principally non-free radical species [7]. To prevent or deter free radical induced lipid oxidation, antioxidants are added to a variety of foods[8]. Substantial tissue injury results, if free radical production rate exceeds the normal capacity of the antioxidant defense mechanisms [9].

Continuous exposure to chemicals and contaminants leads to increase the free radicals amount and causes irreversible oxidative damage including biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to ageing [10,11]. Improved antioxidant status play an important role to minimize the oxidative damage[12].The interest in plant origin natural antioxidants has greatly increased in recent years as the possibility of toxicity of synthetic antioxidants has been criticized [13]. Currently, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the uncontrolled use of modern antibiotics [14-19]. Furthermore there are many reports on antibacterial activity of various plants growing in different region [20,21].

Manilkara zapota L. is an evergreen, glabrous tree under the family *Sapotaceae*. It is cultivated throughout the Indian subcontinent. In Bangladesh the local name is Sofeda. The leaves of this plant are used to treat cough, cold, and diarrhea [22]. For the treatment of diarrhoea and dysentery bark is used. Antimicrobial and antioxidant activities are also reported from the leaves [23,24]. *Polyalthia longifolia* is a lofty evergreen tree under the family *Annonaceae*. It is native to India and in Bangladesh locally it is called Debdaru. This plant is used as an antipyretic agent in indigenous systems of medicine [25]. Bark and leaves of this plant has effective antimicrobial activity [26-28], cytotoxic function [29,30], and hypotensive effects [31] *Abroma augusta* (L) is a plant under family of *Sterculiaceae* and Ulat kambal is the trade name of this plant. Leaves are used to treat uterine disorders, diabetes, rheumatic pain of joints, and headache with sinusitis [32]. Leaves and stem are demulcent and an infusion of fresh leaves and stem in cold water is very efficacious in gonorrhoea [33]. The root-bark is used as an emmenagogue and uterine tonic, the action of dried roots and the sap of the fresh root, has been reported [34]. *Ficus hispida* L. belongs to the family *Moraceae* commonly known as dumoor in Bangladesh. Almost all parts of this plant are used as a folklore remedy for the treatment of various diseases, but the leaves are of particular interest from a medicinal point of view [35] as an antidiarrheal [36] hepatoprotective [37], anti-inflammatory [38], antitussive, antipyretic, astringent, vulnerary, hemostatic and anti-ulcer drug, among other parts [35,39]. *Vitex negundo* L. is a plant under family *Verbenaceae* which is locally known as nishinda in Bangladesh. The leaves and the roots of this plant are important as drugs. Analgesic and anti-inflammatory activities of *Vitex negundo* L. seeds [40,41] and fruits [42] have been reviewed thoroughly. Anti-inflammatory and analgesic properties of mature fresh leaves have also been reported [43].

As part of the endeavor for search of medicinal properties in local floristic resources we herein report a study of antioxidant and antimicrobial activity of the above five medicinal plants.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The plants namely *Manilkara zapota* L., *Polyalthia longifolia*, *Abroma augusta* L., *Ficus hispida* L., *Vitex negundo* L. were collected from Village: Jabra Thana: Dumuria, District: Khulna, Bangladesh in July 2010 and was identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 32767 has been deposited for future reference.

2.2 Chemicals and Drugs

DPPH (1, 1-diphenyl-2-picrylhydrazyl), Ascorbic acid & sodium nitroprusside, sodium phosphate, sulphanilamide, phosphoric acid, naphthyl ethelene diamine were obtained from SD Fine Chem. Ltd. India, ammonium molybdate from Merck, Germany, ferric chloride & neocuproine were obtained from Sigma Chemical Co. USA. Kanamycin and nystatin were collected from Square Pharmaceuticals Ltd., Bangladesh.

2.3 Drying and Pulverization

The fresh leaves of the plants were first washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days. After complete drying, the entire portions were

pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container for further use. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.4 Cold Extraction

About 50 gm of *Manilkara zapota*, 50 gm of *Polyalthia longifolia*, 75 gm of *Abroma augusta* Linn, 100 gm of *Vitex negundo* Linn, 50 gm of *Ficus hispida* Linn powdered material was taken in a clean, flat bottomed 5 glass container and soaked in 500 ml of 95% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

2.5 Extraction with Chloroform

The concentrated methanol extract was made slurry with water. The slurry was taken in a separating funnel and few ml Chloroform (50 ml) was added to the aqueous solution and the mass was shaken vigorously in a separating funnel. Then the funnel was allowed to stand for few minutes for the complete separation of the layers. The organic (lower layer) layer was collected. The process was repeated two times.

2.6 Extraction with Petroleum Ether

After chloroform extraction petroleum ether (50ml) was added to the Methanolic aqueous solution and the mass was shaken vigorously in a separating funnel. Then the funnel was allowed to stand for few minutes for the complete separation of the layers. The organic (upper layer) layer was collected. The process was repeated two times.

The filtrates (chloroform and petroleum ether extract) obtained were concentrated at 50°C under reduced pressure using vacuum pump rotary evaporator (STUART RF3022C, UK). It rendered a gummy concentrate of reddish black color. The extract was transferred to a closed container for further use and protection.

2.7 Determination of Antioxidant Potentials

2.7.1 DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH [44,45]. The absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted and IC₅₀ values were calculated.

2.7.2 Nitric oxide scavenging assay

Nitric Oxide Scavenging assay was carried out according to the procedure described earlier [46]. The method is based on the generation of NO from sodium nitroprusside and subsequent estimation of nitrite ions using Griess reagent produced by the reaction of NO with oxygen in aqueous solution at physiological pH (7.2). In a test tube 4 ml of plant extract or standard of different concentrations were mixed with 1.0 ml of Sodium nitroprusside (5mM) solution. Then the test tube was incubated for 120 minutes at 30°C. After incubation 2

ml of solution was withdrawn from the mixture and mixed with 1.2 ml of griess reagent (1% Sulfanilamide in 5% H₃PO₄ and 0.1% Naphthylethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphaniamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was measured at 550 nm using a spectrophotometer against a blank. The percent (%) inhibition of nitrite formation was calculated from the following equation.

$$\{(A_0 - A_1)/A_0\} \times 100$$

Where A₀ is the absorbance of the Control and A₁ is the absorbance of the extract or standard. The half maximal inhibitory concentration (IC₅₀) was calculated by linear regression method.

2.7.3 Determination of total phenolic content

The content of total phenolic compounds in plant extracts was determined by Folin–Ciocalteu Reagent (FCR) using UV spectrophotometer (UV–1501PC SHIMADZU, Japan) described by the method [47]. 0.5 ml of diluted plant extract and standard of different concentrations solution were taken in the test tube followed by adding 5 ml of Folin – ciocalteu (Diluted 10 fold with water) & 4 ml of Sodium carbonate (1 M) respectively. Solutions were then incubated for 15 minutes at 45°C in the water bath. The absorbance was measured colorimetrically at 765 nm to determine the total phenol content. Various concentrations of Gallic acid (25, 50, 100, and 200 µg/ml) were used to prepare the standard curve and the total content of Phenolic compounds in the crude extracts was calculated according to the following formula:

$$C = (c \times V)/m$$

Where C is the total content of phenolic compounds in mg/g plant extract; c is the concentration of Gallic acid established from the calibration curve in mg/ml; V is the volume of extract in ml and m is the weight of Chloroform or Pet. ether extract in g. The value of total content of phenolic compounds is expressed as GAE (Gallic Acid Equivalent) in mg/g extract [48].

2.7.4 Determination of total flavonoids content

The total flavonoid in the crude extracts was measured using the Aluminum Chloride Colorimetric Method [49]. To 1 ml of plant extract or standard of different concentrations 3 ml Chloroform or Pet. ether, 0.2 ml of 10% aluminum chloride, 0.2 ml potassium acetate (1M) and 5.6 ml of distilled water were added. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using UV spectrophotometer against a blank. Standard curve was prepared using quercetin by dissolving it in Chloroform or Pet. ether followed by serial dilution to 25, 50, 100, 200 µg/ml.

2.7.5 Determination of total antioxidant capacity

The determination of antioxidant capacity in the plant extract was assessed by the phosphomolybdenum method [50]. The method is based on the reduction of Mo (VI)–Mo (V) by means of the extract followed by the formation of a green phosphate/Mo (V) complex at acid pH. A 0.3 ml of crude extract and 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were mixed together. The resulting

solution was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using UV spectrophotometer against a blank after cooling to room temperature. Chloroform or Pet. ether (0.3 ml) instead of plant extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (AAE) and was calculated by the following formula:

$$A = (c \times V)/m$$

Where A is the total content of antioxidant compounds, mg/g plant extract, in Ascorbic acid; c represents the concentration of Ascorbic acid established from the calibration curve, mg/ml; V is the volume of extract in ml and m is the weight of pure plant extract in g.

2.7.6 Reducing power

The reducing power was determined according to the method previously described [51]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe³⁺/Ferricyanide complex to the ferrous form. Therefore, Fe²⁺ can be monitored by measuring the formation of Perle's Prussian blue spectrophotometrically [52]. To 1 ml of crude extract 2.5 ml phosphate buffer (0.2 M, pH 7.0) and 2.5 ml of potassium Ferricyanide K₃Fe (CN)₆ solution, 1% (w/v) was added. The resultant mixture was incubated for 30 minutes at 50°C to complete the reaction and added 2.5 ml (10% w/v) trichloroacetic acid. Then the total mixture was centrifuged at 1800 rpm for 10 min. Further 2.5 ml of the supernatant solution was withdrawn from the mixture and mix with 2.5 ml of distilled water and added 0.5 ml (0.1% w/v) FeCl₃ solution. The absorbance of the solution was then measured at 700 nm using a spectrophotometer against blank. Ascorbic acid and phosphate buffer were used as the standard and blank respectively. Increased absorbance of the reaction mixture represents increased reducing power.

2.8 Antimicrobial Potentials

2.8.1 Antibacterial screening

In vitro antibacterial screening of the crude extracts was carried out by the disc diffusion method [53,54]. Disc diffusion method is equally suited to screening of antibiotics or the products of plant evaluation [55] and is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally the more susceptible the organism, the bigger is the zone of inhibition. In this method the compounds were applied to the agar medium by using paper discs [56,57]. The method is essentially a qualitative or semi quantitative test which allows classification of microorganisms as susceptible, intermediate or resistance to the test materials as well as bacteriostatic or bactericidal activity of a compound [58]. The antibacterial activity was determined against two pathogenic Gram- positive (*Bacillus megaterium*, *Bacillus subtilis*) and six Gram- negative (*Salmonella paratyphi*, *Salmonella typhi*, *Vibrio mimicus*, *Escherichia coli*, *Shigella boydii* and *Aspergillus niger*) bacteria. The extracts were dissolved separately in chloroform or Pet. ether and applied to sterile discs at a concentration of 500 µg/disc and carefully dried to evaporate the residual solvent. Here, Kanamycin 30 µg/disc (Oxoid Ltd., England) was used as the standard. These plates were then kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the organisms. The test material having antibacterial activity inhibited

the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

2.8.2 Antifungal screening

Antifungal activity was determined at a concentration of 500 µg/disc by the disc diffusion method [53,54]. Nystatin 30 µg/disc was used as the standard.

2.8.3 Collection of test organisms

The bacterial species used in the present study were *Bacillus megaterium*, *Bacillus subtilis*, *Salmonella paratyphi*, *Salmonella typhi*, *Vibrio mimicus*, *Escherichia coli*, *Shigella boydii* and *Aspergillus niger*. These were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), Dhaka University and International Center for Diarrheal Disease and Research, Bangladesh (ICDDR) Dhaka, Bangladesh. Tested fungi *Saccharomyces cereviceae*, and *Candida albicans* were collected from the Microbiology research laboratory, Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

3. RESULTS

3.1 DPPH Radical Scavenging Activity

The results of DPPH free radical scavenging activity on the two crude extracts of five plants and of ascorbic acid (standard) are shown in Table 1, where maximum radical scavenging activity (IC₅₀ value, 191.308 µg/ml) was shown by *Polyalthia longifolia*. The minimum radical scavenging activity was shown by MZPE (IC₅₀ value of 63.479 µg/ml), while the IC₅₀ value of Ascorbic Acid was 43.129 µg/ml, which is a well known antioxidant. DPPH radical scavenging activity of the plant extracts are arranged in the following descending order: PLCE > FHCE > VNCE > AACE > MZCE for Chloroform extracts and PLPE > VNPE > FHPE > AAPE > MZPE for Pet ether extracts respectively. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color.

3.2 Nitric Oxide Scavenging Assay

The different extracts of the different plants exhibited dose dependent scavenging of nitric oxide (Table 1) with an IC₅₀ value of (140.700,109.420 µg/ml); (81.331,93.480 µg/ml); (146.520, 80.005 µg/ml);(89.810, 94.206 µg/ml); (54.261, 85.581 µg/ml) respectively for the Chloroform and Pet Ether extracts of *Manilcara zapota* (MZCE, MZPE), *Polyalthia longifolia* (PLCE,PLPE), *Abroma augusta* (AACE, AAPE), *Ficus hispida* (FHCE, FHPE), *Vitex negundo* (VNCE, VNPE) plants compared to 50.171 µg/ ml which was the IC₅₀ value for the reference ascorbic acid. The highest NO scavenging activity was shown by AACE and lowest by VNCE.

Table 1. DPPH radical and NO scavenging activity

Name of the extracts	IC ₅₀ (µg/ml)	
	DPPH Method	NO Method
MZCE	65.714 ± 1.623	140.700 ± 2.027
MZPE	63.479 ± 1.175	109.420 ± 1.648
PLCE	138.235 ± 0.994	81.331 ± 2.186
PLPE	191.308 ± 28.450	93.480 ± 1.271
AACE	75.884 ± 1.857	146.520 ± 3.323
AAPE	64.420 ± 0.159	80.005 ± 0.0721
FHCE	78.667 ± 4.357	89.810 ± 2.213
FHPE	78.044 ± 1.574	94.206 ± 1.322
VNCE	77.563 ± 2.259	54.261 ± 1.458
VNPE	83.625 ± 0.890	85.581 ± 1.347
Ascorbic acid	43.129 ± 1.181	50.171 ± 1.040

Data are expressed as Mean ± SD of triplicate experiments.

3.3 Total Phenol and Total Flavonoids

The results of the phenol and flavonoid content of crude extracts are given in Table 2. The total phenolic contents of plant extracts was determined using the Folin–Ciocalteu assay and was expressed as gallic acid equivalents (GAE). The phenolic content of the studied plant extracts was varied from 0.362 to 180.434 mg/g of the dry weight. Total phenolic content of the plant extracts is arranged in the following descending order: VNPE > VNCE > AAPE > FHCE > MZPE > FHPE > AACE > MZCE > PLPE > PLCE. On the other hand the total flavonoid content of the extracts was evaluated by aluminum colorimetric assay in which quercetin was used as an internal standard where the total flavonoid content ranged from 36.173 to 1265.255 mg/g. Total flavonoid content of the plant extracts is arranged in the following succession: VNPE > VNCE > FHCE > FHPE > AAPE > AACE > MZCE > MZPE > PLPE > PLCE. The Total flavonoid content of *Polyalthia longifolia* is lowest both for Chloroform and Pet ether extracts.

Table 2. Total phenol and total flavonoids content

Name of the extracts	Total phenol (mg/g)	Total flavonoid (mg/g)
MZCE	16.304 ± 2.818	93.316 ± 3.247
MZPE	49.094 ± 2.561	78.265 ± 0.722
PLCE	0.362 ± 0.256	36.173 ± 14.070
PLPE	5.434 ± 1.793	58.112 ± 0.361
AACE	18.659 ± 9.735	198.418 ± 66.021
AAPE	69.927 ± 3.842	219.337 ± 14.792
FHCE	68.115 ± 15.628	1045.612 ± 165.954
FHPE	38.949 ± 14.347	788.214 ± 275.267
VNCE	132.427 ± 69.173	1254.541 ± 16.235
VNPE	180.434 ± 142.19	1265.255 ± 165.593

Data are expressed as Mean ± SD of triplicate experiments.

3.4 Total Antioxidant Activity

Table 3 represents the total antioxidant capacities of the studied plant extracts and expressed as an ascorbic acid equivalents where FHCE showed the highest antioxidant capacity (837.558 mg ascorbic acid/g plant extract) while the cheapest activity was found to be (22.470 mg ascorbic acid/g plant extract) for MZCE.

Table 3. Total antioxidant activity

Name of the extracts	No. of ascorbic acid equivalents (mg/g)
MZCE	22.470 ± 8.016
MZPE	399.651 ± 52.622
PLCE	589.186 ± 129.910
PLPE	525.232 ± 47.688
AACE	797.325 ± 73.999
AAPE	608.721 ± 73.506
FHCE	837.558 ± 110.835
FHPE	816.511 ± 130.732
VNCE	527.267 ± 17.677
VNPE	530.407 ± 88.059

Data are expressed as Mean ± SD of triplicate experiments.

3.5 Reducing Power

Fig. 1 shows the reductive capabilities of the plant extracts compared to Ascorbic acid; determined using the potassium ferricyanide reduction method. The reducing power of the extracts were strong and dose dependent. The activity was found to increase with increasing concentration of the plant extracts and serves as a significant indicator of their potential antioxidant activity. However, MZPE displayed the highest reducing power.

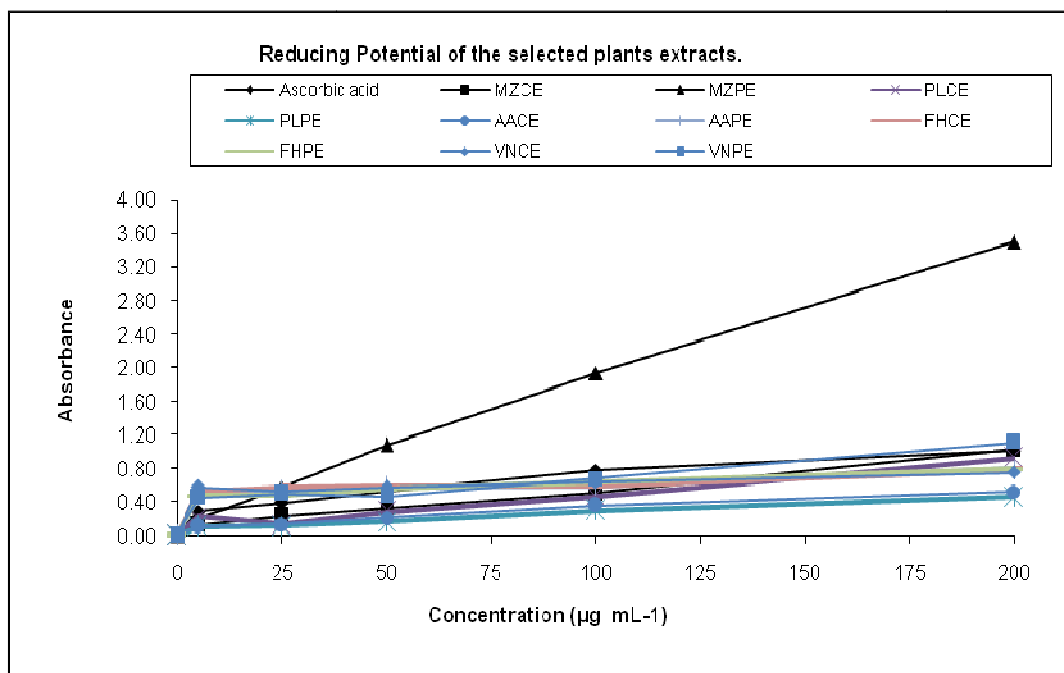


Fig. 1. Reducing power of the selected five plants in chloroform and pet ether extracts

3.6 Antimicrobial Assay

Antibacterial activities of the extracts were tested against eight pathogenic bacteria and were compared with the standard antibiotic Kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in Table 4. PLPE showed highest zone of inhibition up to 40 mm while FHPE showed up to 9 mm which is the lowest zone of inhibition. Among all, *Bacillus megaterium* showed highest sensitivity to extracts and *Salmonella typhi* showed lowest sensitivity. All the plant extracts showed antibacterial activity against all tested Gm (+ve) and Gm (-ve) bacteria. Out of eight bacteria PLPE showed highest antibacterial activity against 5 bacteria while AAPE showed lowest antibacterial activity against 5 bacteria. Antifungal activities of the extracts were tested against two pathogenic fungi and were compared with the standard Nystatin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in Table 4. Between the tested fungi, *Saccharomyces cerevaceae* showed highest zone of inhibition (35 mm) for PLPE and lowest zone of inhibition (8 mm) for AACE.

Table 4. Antibacterial and anti fungal activity

Test organisms	Diameter of zone of inhibition (500 µg/disc)										
	FHP E	AA PE	VNPE	MZP E	PLPE	MZC E	PLC E	AAC E	VNCE	FHCE	Kanamycin (30µg/disc)
Gram Positive											
<i>Bacillus megaterium</i>	13	10	15	20	40	20	30	29	21	16	30
<i>Bacillus subtilis</i>	20	16	24	21	20	19	27	14	17	13	28
Gram Negative											
<i>Salmonella paratyphi</i>	15	16	23	15	30	15	28	25	22	13	25
<i>Shigella boydii</i>	15	11	20	14	29	20	25	23	24	19	33
<i>Vibrio mimicus</i>	16	12	21	18	22	17	23	15	20	22	30
<i>Aspergillus niger</i>	10	11	24	17	28	20	26	15	16	17	30
<i>Salmonella typhi</i>	9	11	25	11	35	17	21	13	23	21	31
<i>E. coli</i>	10	20	18	15	28	20	30	28	25	15	32
Fungi											Nystatin
<i>Candida albicans</i>	17	13	22	17	28	18	25	12	21	18	32
<i>Saccharomyces cerevisiae</i>	16	10	25	18	35	25	30	8	19	11	32

4. DISCUSSION

For investigation of antioxidant potentials of the used plant extracts different *in vitro* antioxidant assays were used. Generally, extracts and/or compounds displaying high antioxidant activity by one method had good antioxidant activity by the other methods and likewise for compounds with low activity [59]. Because no single method can fully evaluate the total antioxidant capacity, different antioxidant compounds may work through different mechanisms. For this reason, multi-method approach is often used for studying the complex antioxidant activities; all methods did not give the same results for activity [60]. Frankel [61] and Warner [62] suggested the antioxidant activity should be measured by using more than one method due to the limitations. In the present study the crude extracts showed different levels of antioxidant activity in different antioxidant techniques employed.

DPPH is a stable free radical potentially reactive with substance able to donate a hydrogen atom and thus useful to assess antioxidant activity of specific compounds of extracts [63]. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized and quantitatively can be measured from the changes in absorbance. Various reports suggest that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines by their hydrogen donating ability reduce and decolorize DPPH [64].

Nitric oxide (NO) or reactive nitrogen species (RNS) are very reactive which are responsible for altering the structural and functional behavior of many cellular components. In some physiological processes like smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity, NO is a potent Pleiotropic mediator [65]. Studies in animal models have suggested a role for NO in the pathogenesis of inflammation and pain and RNS inhibitors have been shown to have beneficial effects on some aspects of the inflammation and tissue changes seen in models of inflammatory bowel disease [66]. NO scavenging capacity of the extracts may help to arrest the chain of reactions initiated by excess generation of NO that are harmful to the human health [67]. In the present study the different extracts of the different plants exhibited dose dependent scavenging of nitric oxide, this may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite.

The phosphomolybdenum method is an alternative to the methods already available for the evaluation of total antioxidant capacity. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid [50]. Total antioxidant activities by the phosphomolybdenum method usually detect antioxidants such as ascorbic acid, some phenolics, α -tocopherol, and carotenoids. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts. The present study reveals that the antioxidant activity of the extracts is in the increasing trend with the increasing concentration of the plant extracts. The antioxidant activity of the plant extracts is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen [50,68].

Polyphenolic compounds (flavonoids, phenolic acids) found in plants have been reported to have multiple biological effects, including antioxidant activity [69,70]. Several studies have also revealed that the phenolic content in the plants are associated with their antioxidant activities, because of their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [49,71,72]. So it is assumed that the high

phenolic content may be responsible for the free radical scavenging activity of the plant extracts.

Reducing power is a convenient and rapid screening method, which may serve as a significant indicator of its potential antioxidant activity [73]. It has been reported that there is a direct correlation between antioxidant capacity and reducing power of certain plant extracts [74]. The reducing properties are generally associated with the presence of reductones [75], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom and may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals [76]. So the ferric reducing property of plant extracts (Fig. 1) implies that they are capable of donating hydrogen atom in a dose dependent manner.

Antimicrobial activity was conducted against a wide range of human pathogenic microorganisms including not only Gram-positive and Gram-negative bacteria, but also fungi. The earlier reports of antimicrobial activities support the findings of present studies. However demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [77,78].

It was observed that different parts of the selected plant extracts in our investigation were studied earlier and exhibited antioxidant and antimicrobial activity to some different extent. In case of *Manilkara zapota*, antimicrobial and antioxidant activities are reported from the leaves which are slightly different in our present study [23,24]. In earlier report there was no response in pet ether and chloroform extract for total phenol and total flavonoid content but present study reports a significant output of these, it may be because of difference in the order of solvents used [24]. Earlier reports also suggested, acetone extract had maximum phenol content and showed good DPPH scavenging activity, thus agreeing with the reports that there is a direct correlation between phenolic content and antioxidant activity [79]. The acetone, ethyl acetate, aqueous extracts showed a concentration dependent reducing capacity and there is an indication that antioxidant properties are concomitant with the development of reducing power [51]. It has been reported that there are many medicinal uses of different parts of *Polyalthia longifolia* plant [27,80-82]. Different chemical constituents were isolated from the leaves of *P. longifolia*, the essential oils of the leaf and stem bark has been studied [83-85]. In earlier methanolic extracts of different parts of *P. longifolia* were tested for their antibacterial and antifungal properties [27]. The antimicrobial activities of the petroleum ether extract of the stem bark of *P. longifolia* were studied [86]. The antioxidant properties of the present study are relevant to the previous report; there is no report on chloroform extract study of this plant but antimicrobial report is also relevant with earlier study. In earlier the *Abroma augusta* extracts were investigated for its antioxidant activity by using hydrogen donation assay method. The methanolic extract of *Abroma augusta* shows strongest antioxidant activity with IC_{50} value of 51.9785 mg/ml [87]. In our present study the IC_{50} value of the chloroform and pet ether extract is 797.325 ± 73.999 and 608.721 ± 73.506 mg/ml which are the indication of very potent antioxidant activity. The n-hexane extract of the seeds of *Abroma augusta* was used as antifungal and the activity was tested against different microorganisms [88].

The present study is the extensive one which reflects that the plant has not only antifungal but also antibacterial activity. The methanol and ethanol extract of stem bark and roots of *Ficus hispida* has shown potent *in vitro* antioxidant activity [89,90]. The current investigation of antioxidant activity of chloroform and pet ether extract of leaves of *Ficus hispida* plants shows potential activities. The hydro alcoholic extract of leaves of *Ficus hispida* was found

effective against *Actinomyces viscosus* [91] but chloroform and pet ether extract of leaves of *Ficus hispida* was found effective against *Bacillus megaterium*, *Bacillus subtilis*, *Salmonella paratyphi*, *Salmonella typhi*, *Vibrio mimicus*, *Escherichia coli*, *Shigella boydii*, *Aspergillus niger* bacteria and *Saccharomyces cerevaceae*, *Candida albicans* fungi. Previous study on antioxidant property of *Vitex negundo* plant showed no significant result [92] but recent study suggests the plant has potential antioxidant activity [93]. The antibacterial activity shown by this plant extract was also reported earlier [94]. So the present study supports the previous report on presence of both the antioxidant and antibacterial activity of this plant, in addition the plant has the potential antifungal activity.

In the present study it was observed that among the extracts the pet ether extracts has the potential Polyphenolic (phenolic acids and flavonoids) contents. It is known that different phenolic compounds have different responses in the Folin-Ciocalteu method. So on the basis of its total phenolic content the antioxidant activity of an extract cannot be predicted [95]. Based on the results it can be concluded that the crude extracts have the potential as antimicrobial compounds against microorganisms and may be utilized in the treatment of infectious diseases caused by resistant organisms.

5. CONCLUSION

The observations found in the present study support this view that the medicinal plants hold a valuable source of potential antioxidants for the discovery of natural-product pharmaceuticals and to be used as preventive agents in the pathogenesis of various diseases. Further works on identification and isolation of active constituents in the extracts may be exploited by *in vivo* study to determine the underlying mechanism of the overall antioxidant activity. On the basis of the obtained results of antimicrobial potentials it can be suggested that, further evaluation of the antibacterial and antifungal properties of the plant extracts against a more extensive panel of microbial agents is reasonable.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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