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Original article

Comparative study among *Avicennia marina*, *Phragmites australis*, and *Moringa oleifera* based ethanolic-extracts for their antimicrobial, antioxidant, and cytotoxic activities

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ABSTRACT

Microbial resistance and other emerging health risk problems related to the side effects of synthetic drugs are the major factors that result in the research regarding natural products. Fruits, leaves, seeds, and oils-based phyto-constituents are the most important source of pharmaceutical products. Plant extract chemistry depends largely on species, plant components, solvent utilized, and extraction technique. This study was aimed to compare the ethanolic extracts of a mangrove plant, i.e., *Avicennia marina* (1E: Lower half of *A. marina*'s pneumatophores, 2E: *A. marina*'s leaves, 3E: Upper half of *A. marina*'s pneumatophores, and 4E: *A. marina*'s shoots), with non-mangrove plants, i.e., *Phragmites australis* (5E: *P. australis*'s shoot), and *Moringa oleifera* (6E: *M. oleifera*'s leaves) for their antimicrobial activities, total phenolic contents, antioxidant activity, and cytotoxicity potential. The antimicrobial activity assays were performed on gram-positive bacteria (i.e., *Bacillus subtilis* and *Staphylococcus aureus*), gram-negative bacteria (i.e., *Escherichia coli*, and *Pseudomonas aeruginosa*), and fungi (i.e., *Aspergillus niger*, *Candida albicans*, and *Rhizopus* spp.). We estimated antioxidant activity by TAC, DPPH, and FRAP assays, and the cytotoxicity was evaluated by MTT assay. The results of antimicrobial activities revealed that *B. subtilis* was the most sensitive to the tested plant extracts compared to *S. aureus*, while it only showed sensitivity to 6E and Imipenem. 5E and 6E showed statistically similar results against *P. aeruginosa* as compared to Ceftazidime. *E. coli* was the most resistant bacteria against tested plant extracts. Among the tested plant extracts, maximum inhibition activity was observed by 6E against *A. niger* (22 ± 0.57 mm), which was statistically similar to the response of 6E against *C. albicans* and 3E against *Rhizopus* spp. 2E did not show any activity against tested fungi. We found that 6E (208.54 ± 1.92 mg g^{-1}) contains maximum phenolic contents followed by 1E (159.42 ± 3.22 mg g^{-1}), 5E (131.08 ± 3.10 mg g^{-1}), 4E (i.e., 72.41 ± 2.96 mg g^{-1}), 3E (67.41 ± 1.68 mg g^{-1}), and 2E (48.72 ± 1.71 mg g^{-1}). The results depict a significant positive correlation between the phenolic contents and the antioxidant activities. As a result, phenolic content may be a natural antioxidant source.

1. Introduction

Avicennia marina (Acanthaceae) is a tropical plant and woody shrub that grows in the inter-tidal zones and coastal areas of tropical and subtropical latitudes (Asaf et al., 2021). Mangrove and mangrove associates are very crucial for the global environment for so many reasons, such as; they provide a shield to coastlines against the diverse impact of natural disasters (McIvor et al., 2012); protect the marine ecosystem by nutrients cycling and trapping, accumulating or degrading the pollutants of terrestrial and aquatic origin (Ando et al., 2001; Kim et al., 2016; Usman et al.,

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2013); support marine life (i.e., fish, shrimps, and crabs, etc.) and wildlife (i.e., mammals, birds, and reptiles, etc.) by providing them food, habitat and breeding space (Shobrak and Aloufi, 2014); serve as the residence of many threatened and endangered species (Norhayati et al., 2009); mangrove plants are the source of medicine, fuel, tannins and construction material for native communities (Bhimba et al., 2012; Kovacs, 1999); they are tolerant or raising of atmospheric carbon dioxide and temperature (Macintosh et al., 2012). Mangroves are necessary to have unique mechanisms or morphological specializations for their survival under harsh conditions. Different types of active metabolites of various biological activities are produced even during stress. Researchers have isolated a variety of compounds from mangroves of commercial and medical importance. Extracts of mangroves have demonstrated human, animal, and plant pathogenic behavior. Polyphenols and tannins are abundant in mangroves. The concentrations of these chemicals are varied seasonally. About 346 active metabolites have been isolated from mangroves (Wu et al., 2008).

Phragmites australis, which belongs to the family Poaceae, is a common reed present in semi-aquatic areas. In Saudi Arabia, it was reported only in Eastern regions, where it is found just before the *Avicennia marina* plantation. However, recently Remesh et al. (2019) also reported this reed in the Southwestern region of Saudi Arabia. It is a perennial and salt-tolerant plant with a widespread root infrastructure (Mal and Narine, 2004). *P. australis* is the source of many chemicals like; Aurantiamide acetate, alpha-D-glucose, benzoic acid, beta-D-glucose, beta-sitosterol, ferulic acid, heptadecanoic acid, methyl gallate, palmitic acid, p-hydroxy, p-coumaric acid, p-hydroxybenzaldehyde, stigmasterol, syringic acid, vanillic acid, 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propa none, (+)-lyoniresinol, and (+)-lyoniresinol-3α-O-β-D-glucopyrano side (Choi et al., 2009; Petropoulos et al., 2018). In traditional medication (Palombo and Semple, 2001), it is used to treat many human and livestock illnesses (González-Tejero et al., 2008; Kiviat and Hamilton, 2001; Viegi et al., 2003). Antioxidant and hepatoprotective properties were demonstrated in aquatic extracts made from *P. australis*'s rhizomes. Further leave extract also possesses anti-melanogenesis and antioxidant activities (Chen et al., 2013; Sim et al., 2017); moreover, Zhu et al. (2017) reported antiviral activity of its water-soluble extracts.

Moringa oleifera is one of the angiospermous plants, which is widely cultivated. It has well renowned nutritional values as it is rich in minerals, proteins, and vitamins. *Moringa oleifera* is the most commonly grown species of the Moringaceae family, mainly native to Himalayan and sub- Himalayan areas. It is grown in Afghanistan, Bangladesh, Pakistan, India, and other tropical and subtropical countries. It is also cultivated in Saudi Arabia (Alaklabi, 2015). Indians and Africans traditionally use *Moringa oleifera* to treat more than 300 diseases, including cancer (Abdel-Daim et al., 2020a; Matic et al., 2018). Moringa's phytoconstituents are recognized for their anti-diabetic, antioxidant, anti-inflammatory (Abd Rani et al., 2018; Abou-Zeid et al., 2021), anti-apoptotic (Abdel-Daim et al., 2020b) and neuroprotectant effects (Kandeil et al., 2019). It contains a variety of beneficial components, including flavonoids, phenols, and vitamin C (ascorbic acid), a-tocopherol, and b-carotenoids, etc. (Alhakmani et al., 2013; Barhoi et al., 2021; Mumtaz et al., 2021; Singhal et al., 2012; Weon et al., 2015).

The essential source of potent antioxidants, medicine, and other pharmaceutical products are natural commodities, especially fruits, leaves, seeds, and oils (Table 1). Secondary metabolites, including phenolic contents, are of particular importance among plant constituents, as they have been reported for the activities of medicinal importance such as antioxidant potency, antibacterial, anticancer, and anti-diabetic activities as well as anti-inflammatory effects (Jeong et al., 2004; Petlevski et al., 2013; Wijngaard et al., 2012). Phenols are the most abundant group in plant extracts. One area where phenols are considered important is to protect the organism against oxidative stress conditions. Oxidative stress is oxidizing/antioxidant disequilibrium in the living organism, in which unstable (free radicals) molecules become stable by electron combinations with other molecules. It is often linked to tissue destruction and other cancer-like chronic diseases (Aslan et al., 2011). Oxidative stresses result from the accumulation of free radicals from ambient sources or generation in the body (Abou-Zeid et al., 2021). Moreover, the antioxidant activities of the extract also rely on the polarity of the solvent, the extraction process, the purity of extraction, and the way the antioxidant is evaluated (Wanasundara and Shahidi, 1998). As a result, any medicinal plant that manages stress-related diseases must have the ability to avoid oxidative stress (Muniyandi et al., 2017).

In this study, we aimed to compare the ethanolic extracts of *Avicennia marina*, *Phragmites australis,* and *Moringa oleifera* with respect to their antimicrobial, antioxidant, and cytotoxic activities.

2. Material and methods

2.1. Sample collection

Plant samples of *Avicennia marina* and *Phragmites australis* were collected randomly from the Eastern Region of KSA along the Arabian Gulf. In contrast, the sample of *Moringa oleifera* was collected from the College of Food and Agriculture Sciences, King Saud University, Riyadh, KSA. The purpose of the moringa plant sample was to compare it with mangrove and non-mangrove semiaquatic (i.e., *Phragmites australis*) concerning the following attributes; 1) Antioxidant activity, 2) Antimicrobial activities, 3) Anticancer activity, and 4) Total Phenolic contents. By following standard procedures, we obtained six extracts (i.e. 1E, 2E, 3E, 4E, 5E, and 6E). Sampling details and coordinate of respective sample locations are mentioned in Table 2.

Plant parts were collected and put into sterilized plastic bags using a sterilized scissor/plant cutter. Leaves were taken from the middle part of the branches. Pneumatophores (Aerial roots) were collected in two parts separately, i.e., Upper half and Lower Half. Samples were collected about 1 kg for each plant part. Samples were kept in an ice-box for transportation.

2.2. Extraction process

In the lab, plant parts were washed with sterilized distilled H_2O and 70% ethanol for cleaning and disinfection. Then, without using an oven, air-dried in the shade. Dry samples were then pulverized using a plant grinder (MF 10 basic, IKA® WERKE). The mashed samples were packaged and wrapped in paper before being placed in clean plastic bags. The maceration procedure followed, in which five parts of the solvent were used for one part of the sample. The maceration period lasted five days with regular shaking. The solvent used in this research was absolute ethanol (Haq et al., 2011; Takarina et al., 2018). The extract solutions were evaporated using a rotary evaporator (RV 10C S99, IKA®) to obtain the crude plant extract. The finished plant extracts were kept refrigerated in sterile Eppendorf tubes for future use (Ahmad et al., 2018).

2.3. Estimation of antimicrobial activity

Antimicrobial activity was estimated by the agar plate well diffusion assay (Perez et al., 1990), which was later reported by Mathabe et al. (2006). On Muller-Hinton agar plates, 0.1 ml of diluted culture (10^5 CFU ml^{-1}) of tested organisms were distributed, respectively. In the agar plates ells, wells with a diameter

Table 1
Examples of some reported sources for plant extracts, their active group and their activity.

Plant Source	Active group	Use and effects	Reference
Plectranthus sp. (**Lamiaceae**)	Antioxidants Abietane diterpenoids Flavonoids Glycosides Phenols Steroids Tannins	Antimicrobial anti-inflammatory Antidiabetic Anxiolytic Antineoplastic Analgesic Antimalarial Diuretic Wound healing Skincare Respiratory disorders Antiplatelet aggregation activity	(Cook, 1995; Abdel-Mogib et al., 2002; Permana et al., 1994)
Xanthoria parietina *Lobaria pulmonaria* and *Parmelia sulcata* (**Lichens**)	Ddepsides Depsidones Dibenzofurans Xanthones Terpene derivatives Alcohol	Human nutrition Animal nutrition Pigments/Colours Perfumes Antiviral Antibiotic Antitumor Allergenic Enzyme inhibitory Antioxidant Treatment of jaundice, pulmonary and cranial diseases	(Huneck, 1999; Karagouml et al., 2009; KIRMIZIGÜL et al., 2003; Malhotra et al., 2008; Ranković et al., 2011; Seymour et al., 2005)
Tragopogon porrifolius (**Asteraceae**)	Mono-unsaturated and essential fatty acids Antioxidants Vitamins Polyphenols Flavonoids	Traditional medicine Anticancer	(ACIKARA et al., 2013; Al-Rimawi et al., 2016; Formisano et al., 2010; Pham-Huy et al., 2008; Servili et al., 2002; Silva et al., 2006; Tenkerian et al., 2015)
Hibiscus asper (**Malvaceae**)	Antioxidants	Antiapoptotic Neuroprotective	(Foyet et al., 2011)
Tectona grandis (**Lamiaceae**)	Quinones Naphthotectone Anthratectone 5-hydroxylapachol	Antibacterial	(Khan and Mlungwana, 1999; Lacret et al., 2011; Neamatallah et al., 2005)
Ficus sp. (**Moraceae**)	Monoterpenes Diterpenes and triterpenes Steroids Furocoumarins Pigments Amino acids Alkaloids	Antimicrobial Antidiabetic Anti-inflammatory Analgesic Antiseizure Anti-Parkinson's diseases Cytotoxic Antioxidant	(Annan and Houghton, 2008; Ayim et al., 2007; Salehi et al., 2020; Watcho et al., 2011)
Pentadesma butyracea (**Clusiaceae**)	Cratoxylone, a-mangostin, 1,3,5-trihydroxy-2-methoxyxanthone, Garcinone E Epicathechin Lupeol	Cytotoxic Antimalarial	(Lenta et al., 2011; Zelefack et al., 2009)
Vismia laurentii (**Hypericaceae**)	Xanthones Anthraquinones Naphtoquinones	Anticancer Antimicrobial Antimalarial	(Kuete et al., 2011; Nguemeving et al., 2006; Noungoue et al., 2008; Noungoue et al., 2009; Wabo et al., 2007)
Paullinia pinnata (**Sapindaceae**)	Triterpenoids Flavonoid Polyphenol Proanthocyanidin	Normal cell proliferation Fibroblast Antioxidant Induce arterial relaxation	(Annan et al., 2010; Zamble et al., 2006)
Dichrostachys glomerata (**Fabaceae**)	Alkaloids Phenols Tannins	Antibacterial	(Fankam et al., 2011)
Psorospermum febrifugum (**Hypericaceae**)	Xanthones Emodin	Antiviral Anticancer Antimicrobial	(de Dieu Tamokou et al., 2013; Kisangau et al., 2007; Liu et al., 2012; Permana et al., 1994; Su et al., 2010; Tsaffack et al., 2009)

of 0.8 cm were punched and filled with 0.1 ml of 1000 μg ml^{-1} plant extract and DMSO as a blank treatment. At 37 °C, the plates were incubated overnight. The antimicrobial response was estimated by observing the inhibition zone (mm) against tested organisms. As a positive control, standard drugs (i.e., Imipenem against gram-positive, Ceftazidime against gram-negative, and Nystatin

Table 2
Treatment's description.

Plant Extract	Plant Source	Plant Part	Sampling Location	
			Latitude	Longitude
1E	*Avicennia marina* (Forssk.) Vierh.	Lower half of Pneumatophores	26°34′3.23″N	50° 5′20.80″E
2E	*Avicennia marina* (Forssk.) Vierh.	Leaves	26°34′3.23″N	50° 5′20.80″E
3E	*Avicennia marina* (Forssk.) Vierh.	Upper half of Pneumatophores	26°34′3.23″N	50° 5′20.80″E
4E	*Avicennia marina* (Forssk.) Vierh.	Shoots	26°34′3.23″N	50° 5′20.80″E
5E	*Phragmites australis* (Cav.) Trin. ex Steud.	Leaves and Stem	26°35′39.66″N	50° 3′37.44″E
6E	*Moringa oleifera* (L.)	Leaves	24°43′28.79″N	46°37′4.00″E

against fungi) against respective strains at the concentration of 30 μg ml^{-1} were also tested. Each treatment was repeated three times.

2.4. Estimation of MIC

MIC (Minimum inhibitory concentration) is elaborated as the minimum concentration of plant extracts that inhibited the observable growth of tested microbial strains. The broth dilution technique was used to estimate the MIC of plant extracts against bacteria and fungi. In this method, p-iodonitro tetrazolium violet (0.02 mg ml^{-1} concentration) is used as a growth indicator (Eloff, 1998). For the serial dilutions of plant extracts, 2 ml plant extract was combined with 2 ml Muller-Hinton to get desired dilutions. Before incubation overnight at 37 °C, 2 ml of a fresh culture of tested microbe was added. After visually inspecting turbidity, each tube was applied with 0.8 ml of indicator dye and put in the incubator at 37 °C for 30 min. After incubation, the tubes were observed for color development, and the growth inhibition was also crosschecked by re-culturing 100 μl of culture from the tested tube on nutrient agar.

2.5. Estimation of total phenolic contents

The Folin-Ciocalteau reagent technique was used to calculate the total phenolic contents (TPC) of the plant extracts examined, as reported by Zahin et al. (2013). In short, 2.5 ml of Folin-Ciocalteau reagent (1/10th strength) along with 2 ml of sodium carbonate solution (7.5%, w/v) were mixed into 0.5 ml of each extracted sample and put in the incubator at 45 °C for the duration of 15 min at. Three replications were maintained for each study. After incubation, the absorbance of each sample was observed at the wavelength of 765 nm by Genesys™ 5 spectrophotometer (Thermo Spectronic, USA). The TPC were presented as milligrams of gallic acid equivalent per gram of dry plant extract.

2.6. Estimation of antioxidant potential

The antioxidant capacity of plant extracts was estimated using three different methods; the DPPH test, FRAP assay, and phosphomolybdenum technique, as detailed below;

2.7. DPPH free radical scavenging assay

A slightly modified technique, as stated by Gyamfi et al. (1999), was used to evaluate the free radical activity of various plant extracts against stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical. It is decreased if DPPH interacts with an antioxidant present in the tested sample that might provide hydrogen. The color shift from deep-violet to light-yellow was detected by a Genesys™ 5 Spectrophotometer (Thermo Spectronic, USA) at 517 nm. The different concentrations of tested plant extracts were prepared in ethanol, and 50 μl of respective concentration was blended with 1 ml of DPPH solution in methanol (0.1 mM) along with 450 μl Tris-HCl buffer (50 mM, pH 7.4) and incubated at room temperature for 30 min. After incubation, the reduction of the free radical was observed by spectrophotometer. Ethanol was used as a negative control, while butylated hydroxyl toluene (BHT) and ascorbic acid were tested as a positive control in this trial. Inhibition percentage was estimated by following the equation given below:

$$\%\ Inhibition = \frac{Absorbance\ of\ Control - Absorbance\ of\ test\ sample}{Absorbance\ of\ Control} \times 100$$

2.8. Ferric ions (Fe^{3+}) reducing antioxidant power assay

Antioxidant power was determined by observing absorbance resulting from Perl's Prussian blue complex formation following the addition of excess ferric ions (Fe^{3+}) with samples in the form of Fe^{3+} $(CN^{-})_6$, which was subsequently reduced to $Fe^{2+}(CN^{-})_6$. Hence, it is named as the ferric reducing antioxidant power assay or simply FRAP assay as reported by Oyaizu (1986); and later adopted by Gülçin (2009). Plant extracts of various concentrations were prepared in 0.75 ml of distilled water and then mixed with 1.25 ml of sodium phosphate buffer (0.2 M) and 1.25 ml of Potassium hexacyanoferrate (III) (1%). The solution was then put in the incubator at 50 °C for a duration of 20 min, and this mixture was acidified by adding 1.25 ml of Trichloroacetic acid (10%). Subsequently, 0.5 ml of Iron (III) chloride (0.1%) was added to this solution, and the absorbance was observed by using a Genesys™ 5 spectrophotometer at a wavelength of 700 nm (Thermo Spectronic, USA). The absorbance measurement of the sample is directly related to the sample's reduction capacity.

2.9. Phosphomolybdenum assay

The total antioxidant capacity (TAO) of studied plant extracts was determined using the Phosphomolybdenum test as described by Prieto et al. (1999). Known quantity (0.1 ml) from the desired concentration of plant extracts was mixed with 1 ml of reaction reagent, which consists of Ammonium molybdate (4 mM), Sodium phosphate (28 mM) and Sulphuric acid (0.6 M). As a control (Blank), 0.1 ml of ethanol was utilized. The tubes were enclosed and put in the water bath at 95 °C for the duration of 90 min and then cooled down to room temperature. The absorbance readings for each solution were taken at the wavelength of 695 nm against the blank by using Genesys™ 5 spectrophotometer (Thermo Spectronic, USA). Further, the TAO of the tested plant extracts were presented as equivalents to ascorbic acid (μmol g^{-1}).

2.10. Cell viability assay/MTT assay

The influence of plant extracts on the viability of HepG2 cell lines was estimated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) technique, which was reported by Hasan et al. (2016). In 24-well microtiter plates, 10^4 cells were seeded per well with 1 ml of culture media containing extracts of various concentrations (i.e., 0–400 μg ml^{-1}). These

seeded plates were put in the humidified incubator at 37 °C for a duration of 48 h, followed by the addition of 0.2 ml of MTT (5 mg ml^{-1} PBS) per well and plates were incubated again for two hours. Following incubation, 0.2 ml of DMSO was poured into each well and centrifuged (1800*g*, 5 min, and 4 °C). The OD readings, at the wavelength of 540 nm (Shi et al., 2021), were observed by a microplate reader (ELx800, BioTek, US). Cytotoxic activity of extracts was estimated as percent cell viability against the cells treated only with DMSO as control by the equation given below;

$$\%\ Cell\ \ viability = \frac{Absorbance\ \ of\ \ sample - Absorbance\ \ of\ \ blank}{Absorbance\ \ of\ \ DMSO - Absorbance\ \ of\ \ blank} \times 100$$

2.11. Statistical analysis

All measurements were taken three times for each treatment. Data were subjected to the analysis of variance (ANOVA) using a split-plot. Means were compared by Tukey's honestly significant difference (HSD) test ($p \leq 0.05$) in R programming using the agricolae package. The Pearson's correlation among total phenolic content, cell viability assay, and antioxidant activities was evaluated by R programming using the corrplot package.

3. Results

3.1. Antimicrobial activity

The ethanolic based different plant extracts (i.e., 1E: Lower half of *Avicennia marina*'s pneumatophores, 2E: *Avicennia marina*'s leaves, 3E: Upper half of *Avicennia marina*'s pneumatophores, 4E: *Avicennia marina*'s shoots, 5E: *Phragmites australis*'s shoot, and 6E: *Moringa oleifera* 's leaves) were evaluated for their antimicrobial potential against gram-positive bacteria (i.e., *Bacillus subtilis* and *Staphylococcus aureus*), gram-negative bacteria ((i.e., *Escherichia coli*, and *Pseudomonas aeruginosa*), and fungi (i.e., *Aspergillus niger*, *Candida albicans*, and *Rhizopus* spp.) (Figs. 1–3).

In the case of gram-positive bacteria, the commercial control (Imipenem, tested earlier by Buckley et al. (1992)) showed a maximum inhibition zone compared to tested plant extracts. *Bacillus subtilis* was the most sensitive to tested plant extracts compared to *Staphylococcus aureus*, while *Staphylococcus aureus* only showed sensitivity to 6E and Imipenem (as a commercial control). The maximum inhibition zone 16 ± 1.53 mm and 16 ± 1.15 mm was observed by 6E against *Staphylococcus aureus*, and *Bacillus subtilis*, respectively, which was statistically similar to the response of all other tested plant extracts against *Bacillus subtilis* (Fig. 1).

In the case of gram-negative bacteria, the commercial control (Ceftazidime) showed maximum inhibition zone as compared to tested plant extracts, while the response of Ceftazidime (i.e., 20 ± 0.58 mm) was statistically similar to 5E (i.e., 16 ± 1.0 mm)

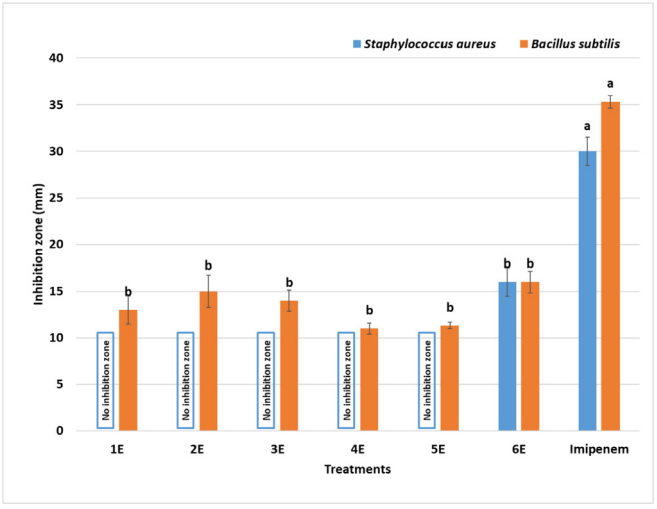

Fig. 1. Antibacterial activity in terms of inhibition zone (mm) resulted by tested plant extracts against gram positive bacteria (i.e. *Staphylococcus aureus*, and *Bacillus subtilis*). Imipenem was used as the control. 1E, 2E, 3E, 4E and 5E did not show any inhibitory effects on the *Staphylococcus aureus*. Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 6.547692).

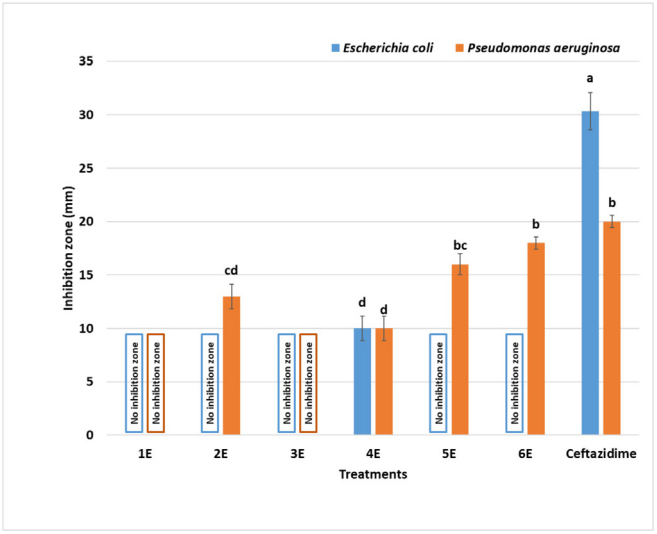

Fig. 2. Antibacterial activity in terms of inhibition zone (mm) resulted by tested plant extracts against gram negative bacteria (i.e. *Escherichia coli*, and *Pseudomonas aeruginosa*). Ceftazidime was used as the control. 2E and 3E did not show any inhibitory effects against both bacteria while 2E, 5E and 6E did not show any inhibitory effects against Escherichia coli. Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 4.463518).

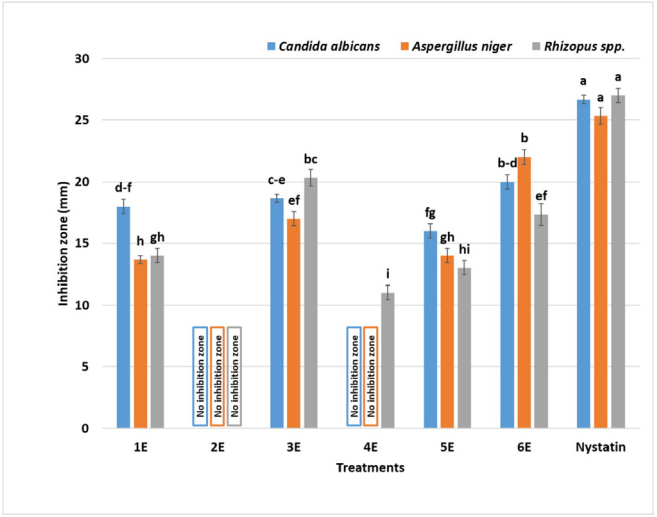

Fig. 3. Antifungal activity in terms of inhibition zone (mm) resulted by tested plant extracts against *Candida albicans*, *Aspergillus niger*, and *Rhizopus* spp. Nystatin was used as the control. 2E did not show any inhibitory effects against all tested fungal strains while 4E showed activity only against *Rhizopus* spp. Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 2.230805).

and 6E (i.e., 18 ± 0.58 mm), against *Pseudomonas aeruginosa*. 1E and 3E did not depict any inhibition activity against any tested bacteria (Fig. 2).

In the case of fungi, the commercial control (Nystatin) showed a maximum inhibition zone compared to tested plant extracts. Among the tested plant extracts, a maximum inhibition zone (i.e., 22 ± 0.57 mm) was observed by 6E against *Aspergillus niger*, which was statistically similar to the response of 6E against *Candida albicans* (i.e., 22 ± 0.58 mm) and 3E against *Rhizopus* spp. (i.e., 20.33 ± 0.67 mm). 2E did not show any inhibition activity against any of the tested fungi (Fig. 3).

3.2. Minimum inhibitory concentrations (MIC)

MIC was tested to estimate the susceptibilities of tested microbes for ethanolic extracts of different plant samples (i.e., 1E: Lower half of *Avicennia marina*'s pneumatophores, 2E: *Avicennia marina*'s leaves, 3E: Upper half of *Avicennia marina*'s pneumatophores, 4E: *Avicennia marina*'s shoots, 5E: *Phragmites australis*'s shoot, and 6E: *Moringa oleifera* 's leaves) and also to compare the potential of used extracts (Figs. 4–6).

1E showed the lowest MIC for *Rhizopus* spp. (i.e., 0.48 ± 0.10 m g ml^{-1}); 3E showed the lowest MIC for *B. subtilis, C. albicans, A. niger, and Rhizopus* spp. (i.e., 0.56 ± 0.13 mg ml^{-1}, 0.56 ± 0.12 mg ml^{-1}, 0.56 ± 0.13 mg ml^{-1}, and 0.48 ± 0.10 mg ml^{-1} respectively); 5E showed the lowest MIC for *C. albicans* (i.e, 0.56 ± 0.13 mg ml^{-1}), while 6E showed lowest MIC for all of tested microorganisms i.e., *S. aureus, B. subtilis, E. coli, P. aeruginosa, C. albicans*, *A. niger*, and *Rhizopus* spp. (i.e., 0.56 ± 0.13 mg ml^{-1}, 0.56 ± 0.12 mg ml^{-1}, 1.6 ± 0.16 mg ml^{-1}, 0.56 ± 0.13 mg ml^{-1}, 0.48 ± 0.10 mg ml^{-1}, 0.56 ± 0.13 mg ml^{-1}, and 0.48 ± 0.10 mg ml^{-1} respectively).

3.3. Antioxidant activities

In this study, antioxidant activities of different plant samples (i.e., 1E: Lower half of *Avicennia marina*'s pneumatophores, 2E: *Avicennia marina*'s leaves, 3E: Upper half of *Avicennia marina*'s pneumatophores, 4E: *Avicennia marina*'s shoots, 5E: *Phragmites australis*'s shoot, and 6E: *Moringa oleifera*'s leaves) were evaluated by phenolic contents (mg g^{-1} of plant extract), total antioxidant capacity (μmoles g^{-1} of extract), free radical scavenging activity

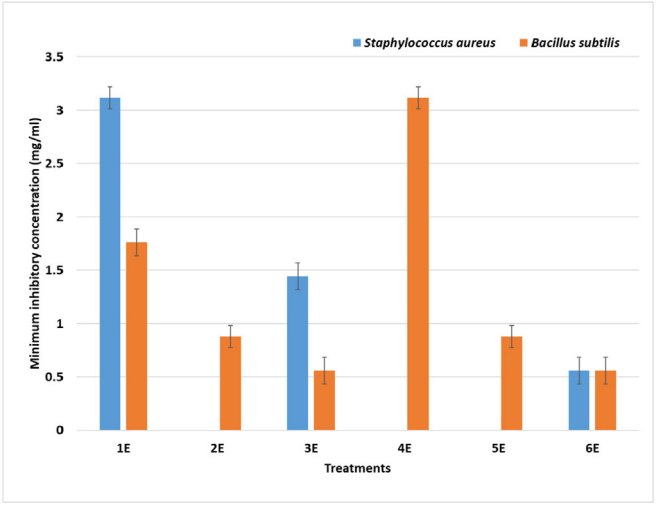

Fig. 4. Minimum inhibitory concentration (mg/ml) values for tested plant extracts against gram positive bacteria (i.e. *Staphylococcus aureus*, and *Bacillus subtilis*). 2E, 4E, and 5E did not show any inhibitory effects on the *Staphylococcus aureus*. Vertical bars show standard error (n = 5).

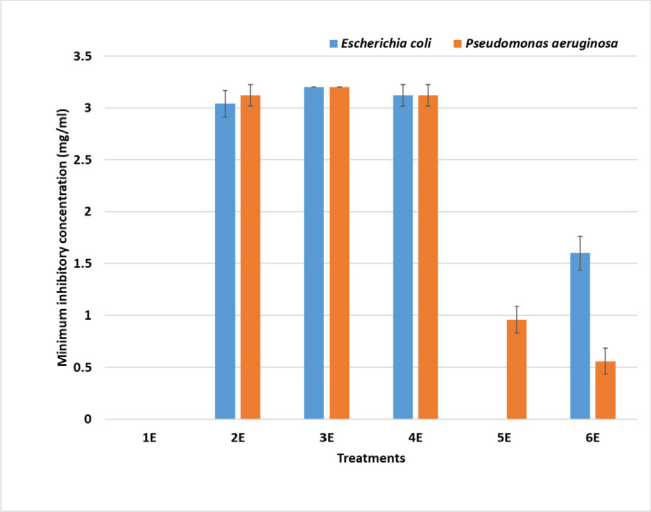

Fig. 5. Minimum inhibitory concentration (mg/ml) values for tested plant extracts against gram negative bacteria (i.e. *Escherichia coli*, and *Pseudomonas aeruginosa*). 1E did not show any inhibitory effects on any of tested bacteria while 5E showed inhibitory effect only against *Pseudomonas aeruginosa*. Vertical bars show standard error (n = 5).

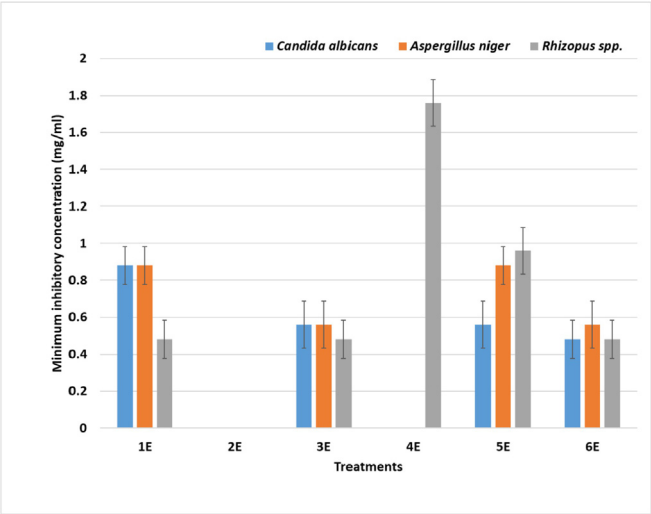

Fig. 6. Minimum inhibitory concenteration (mg/ml) values for tested plant extracts against *Candida albicans*, *Aspergillus niger*, and *Rhizopus* spp. 2E did not show any inhibitory effects on any of tested fungi while 4E showed inhibitory effect only against *Rhizopus* spp. Vertical bars show standard error (n = 5).

(percent decolorization) and the reducing power (absorbance at 700 nm).

The results (Fig. 7) revealed that the total phenolic contents varied significantly in tested extracts. 6E carried the highest phenolic contents (i.e., 208.54 ± 1.92 mg g^{-1} plant extract), while 2E carried the lowest phenolic contents (i.e., 48.72 ± 1.71 mg g^{-1} of plant extract). 1E contains significantly more phenolic contents (i.e., 159.42 ± 3.22 mg g^{-1} plant extract) than 5E (i.e., 131.08 ± 3.10 mg g^{-1} plant extract), while 4E, 3E, and 2E contains significantly less phenolic contents as compared to 5E (i.e., 72.41 ± 2.96 mg g^{-1} plant extract, 67.41 ± 1.68 mg g^{-1} plant extract, and 48.72 ± 1.71 mg g^{-1} plant extract, respectively).

The results of total oxidant capacity (μmoles g^{-1} plant extract) by phosphomolybdenum method (as shown in Fig. 8) depict that 1E showed maximum total antioxidant capacity (i.e., 740.71 ± 159.09 μmoles g^{-1} plant extract), while 2E showed the

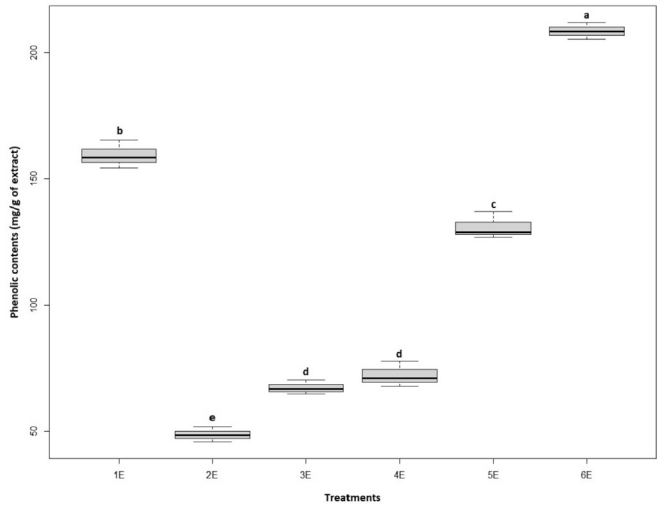

Fig. 7. Phenolic contents (mg/g of extract) of tested plant extracts. Boxplots show the third quartile and first quartile (box edges), median (middle line) and range of the data (whiskers). Each boxplot represents the average of three samples. Boxplots sharing the same letters are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 11.978).

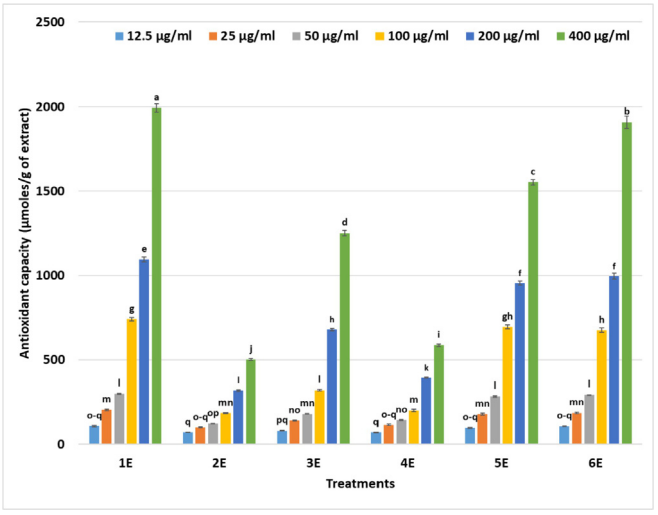

Fig. 8. Total antioxidant capacity of tested plant extracts (µmoles/g of extract) by phosphomolybdenum method at different concentrations (µg/ml). Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 48.47146).

lowest total antioxidant capacity (i.e., 216.96 ± 36.57 µmoles g^{-1} plant extract). As like phenolic contents, 4E, 3E, and 2E showed significantly low total antioxidant capacity as compared to 5E (i.e., 251.29 ± 43.93 µmoles g^{-1} plant extract, 442.28 ± 99.70 µmoles g^{-1} plant extract, and 216.96 ± 36.57 µmoles g^{-1} plant extract, respectively).

The results of the DPPH bioassay (Fig. 9) depict the same trend as phenolic contents except here in the DPPH bioassay results 2E, 3E, and 4E are statistically similar to each other with minimum activity, while 1E and 6E are statistically similar to each other with maximum activity. The reducing power activity of tested ethanolic plant extract samples was carried out by the FRAP method. The results regarding reducing power activity (Fig. 10) also depict the same trend as phenolic contents except here in FRAP bioassay results 2E, 3E, and 4E are statistically different from each other, while 1E and 5E are statistically similar to each other.

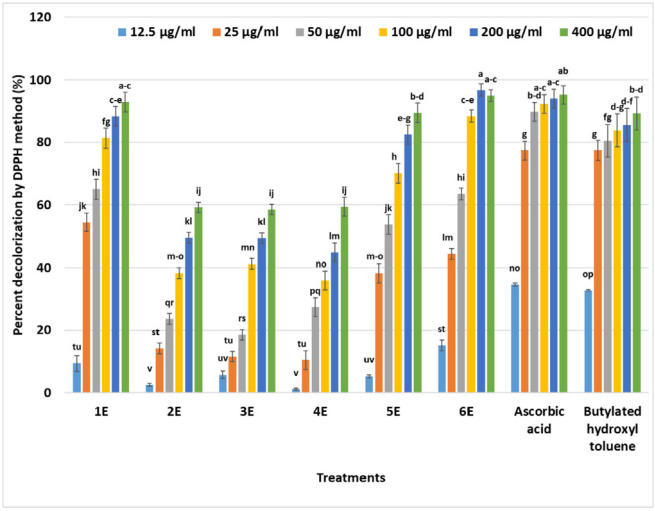

Fig. 9. Free radical scavenging activity of tested plant extracts in terms of percent decolorization (%) by DPPH method. Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 6.689342).

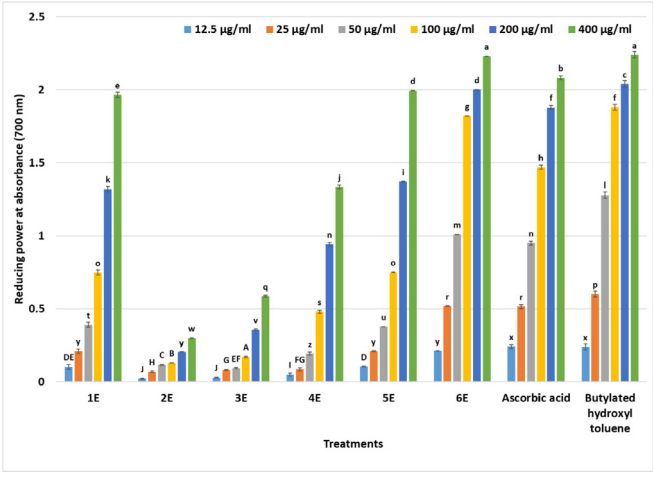

Fig. 10. The reducing power activity of tested plant extracts by FRAP method (700 nm absorbance) at different concentrations (µg/ml). Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test (HSD value = 0.01039239).

3.4. Cell viability assay/MTT assay

HepG2 cancer cells were seeded along with different serial concentrations (0 µg ml^{-1} as a control, 25 µg ml^{-1}, 50 µg ml^{-1}, 100 µg ml^{-1}, 200 µg ml^{-1}, and 400 µg ml^{-1}) of ethanolic plant extracts (i.e., 1E: Lower half of *Avicennia marina*'s pneumatophores, 2E: *Avicennia marina*'s leaves, 3E: Upper half of *Avicennia marina*'s pneumatophores, 4E: *Avicennia marina*'s shoots, 5E: *Phragmites australis*'s shoot, and 6E: *Moringa oleifera*'s leaves) for 48 h. Doses of tested plant extracts, inducing 50% cell growth inhibition (IC_{50}) against HepG2 cancer cells growth are presented in Fig. 11.

4. Discussion

4.1. Antimicrobial activity

A number of plants have been studied for their antimicrobial activities, and extracts of many medicinal plants have been shown

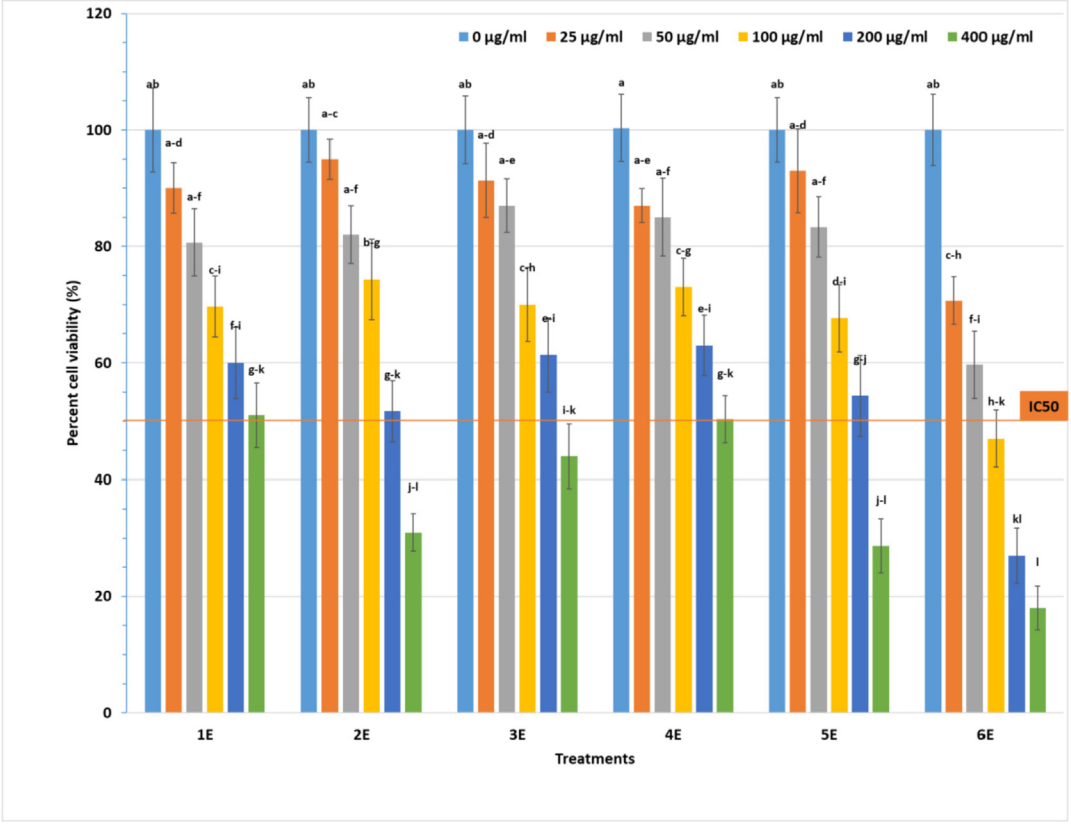

Fig. 11. Cytotoxicity (MTT) assay for tested plant extracts at different concentrations (µg/ml) against HepG2 cancer cell lines after 48 h of incubation. Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test. IC50 indicating the doses of tested plant extracts, inducing 50% HepG2 cancer cell growth inhibition (HSD value = 25.90606).

to be highly efficient against different microbial strains (Mahady et al., 2008). Antibacterial activity may be attributed to active components found in plant extracts. Many studies have shown the potential of mangrove extracts against pathogenic microbes, and they proposed that the mangrove plants are an important source for the bioactive phyto-constituents with enormous medicinal potential (Gurudeeban et al., 2015; Mouafi et al., 2014; Thatoi et al., 2014).

The most common mechanisms for the treatment of microbial infections are; interaction between drug and enzymatic system, inhibition in genome replication, interference with mechanisms associated with normal functions of the cell membrane and cell wall (Mukhopadhyay and Peterson, 2006; Tenover, 2006). Okla et al. (2021) reported the Benzoic acid and Phthalate in *A. marina* based the ethanolic-extracts, which have been shown a permeability barrier provided by the cells membrane and participate in many cellular functions (Baek et al., 2015). Phytol is present in the leaves, and it is the part of chlorophyll that may be transformed to its derivative, i.e., phytanic acid. The antibacterial behavior of leaf extract could be due to phytol (phytanic acid). Stigma-sterol is another compound present in *A. marina*, and it has been stated to participate in lactamase inhibition, which results in sensitivity of the resistant strains to antibiotics (Yenn et al., 2017). The differential antibacterial activity is caused by active components contained in plant extracts. For example, for survival in serious environmental circumstances, *A. marina* accumulated several phytochemicals (Jia et al., 2004; Khattaba and Temraz, 2017; Li et al., 2010; Mahera et al., 2011; Mahera et al., 2013; Okla et al., 2019). 4,5,7-trihydroxyflavone (flavonoids) is the main antioxidant ingredient in methanolic plant extract of *A. marina* along with other secondary metabolites, i.e., alkaloids, flavonoids, and steroids (Thatoi et al., 2014), which may exert antibacterial activity against tested microbial strains (Abeysinghe et al., 2003). Wu et al. (2008) studied the mangrove plants in detail and reported 349 types of metabolites associate with mangroves in terms of species, chemical composition, and their activities. Phyto-constituents work more effectively when used synergistically than individually, as literature also showed that the antimicrobial activity of essential oils as a whole was found to be higher than its single constituents tested separately (EL Moussaoui et al., 2021; Yu et al., 2020).

Our results also depict that all the ethanolic extracts used in this study did not showed antimicrobial potential against all tested microbial strains but they showed antimicrobial activity against at least one tested microbial strains, indicating that ethanol is a good solvent for the extraction of active ingredients of plant samples. The active constituent in extracts results in the creation of microbial growth inhibition that appears as clear areas around the wells. These effects are mainly depending upon the plant species, plant parts, the extraction method, and the solvent utilized for extraction. The Okla et al. (2021) found antibacterial activity in alcohol and chloroform-based extracts of mangrove's root while using ethyl acetate as a solvent only leaves extract showed antimicrobial activities. It clearly depicts that the effectiveness of plant extracts also depends upon parts of the plant used and the solvent utilized for extraction.

However, none of the tested extracts exhibit antibacterial activity against all microbial strains. The resistance response of tested microbes was variable against extracts. These resistant microbes may have certain types of tolerance mechanisms against plant extracts, e.g., decrease the intracellular accumulation of active

ingredients, enzymatic inactivation, the mutation in target sites, and or the dosage of active ingredient may not be appropriate (Okla et al., 2021).

4.2. Antioxidant activity

The current investigation has proven that ethanol as a solvent for the extraction of phyto-constituents can play an important role in their antimicrobial and antioxidant properties. The findings of Haq et al. (2011) clearly showed that the solvent used for extraction significantly influences the total phenolic contents of plant extracts as phenolic contents in chloroform-based plant extract of *B. gymnorrhiza* were ten times the phenolic contents present in methanol and ethanol extract of the same plant sample. While studying *Lentinus edodes* and *Volvariella volvacea*, Cheung et al. (2003) found that for the extraction of phenolic contents, methanol is a more efficient solvent as compared to water ethyl acetate and petroleum ether.

The difference in phenolic contents of different plant extract used in this study might be related to the plants type and plant part difference. As previously reported that the total phenolic contents of plant extracts are influenced greatly by plant type. Kubota et al. (2000) reported that total phenolic components in peach are influenced by its cultivars. Ahmed and Beigh (2009) also stated variation in phenolic contents within subspecies of *Brassica oleracea* var. acephala. Comparison among extracts of different parts of mangrove plants with respect to phenolic contents was also studied earlier by Banerjee et al. (2008), and they concluded that stem and roots of *Ceriops decandra* (Perr.) possess a higher amount of phenolic contents compared to the leaves. In this study, we also found a similar trend that phenolic contents in ethanolic extracts of shoots and aerial roots were higher as compared to leaves of *A. marina*. The study of Haq et al. (2011) also found the higher phenolic compound in barks compared with leaves while studying *Bruguiera gymnorrhiza*.

Phenolic contents show different types of physiological characteristics, such as anti-allergenic, anti-artherogenic, anti-inflammatory (Middleton et al., 2000), antimicrobial (Puupponen-Pimiä et al., 2001), antioxidant (Banerjee et al., 2008), antithrombotic, cardioprotective, and vasodilatory effects (Manach et al., 2005). To control the level of ROS for cell protection from stresses, mangrove tissues are enriched with non-enzymatic antioxidants of low molecular weight like; ascorbate, phenolic compounds, and tocopherols (Thatoi et al., 2014). Estimation of antioxidant activity by one antioxidant assay alone is not recommended (Rafat et al., 2010). Therefore, to get more reliable data, we estimated antioxidant activity by three different assays, i.e., total antioxidant capacity, DPPH method, and FRAP assay. The importance of evaluating antioxidant activity by a combination of different methods is also confirmed by the findings of Haq et al. (2011) and Hakiman and Maziah (2009). The tested extracts in this study showed antioxidant activity correlated to their total phenolic contents (TPC). There was a significant positive correlation among the TPC, cell viability, and their antioxidant activities (Fig. 12). Phenolic compounds could be a key factor for the antioxidant activity of natural products (Kumar et al., 2021; Parr and Bolwell, 2000), and as a result, they may be a natural antioxidant source. Similar correlation trends were also reported by Sethi et al. (2020) while studying apple fruit extracts.

4.3. Cytotoxic activity

Cancer is an emerging health issue around the world. Natural products have long been used to cure and prevent many diseases, including cancer, and thus phyto-chemicals are a good choice for the development of anticancer drugs (Smith-Warner et al., 2000; Tavakkol-Afshari et al., 2008). In the current study, the cytotoxic effects of ethanolic plant extracts in HepG2 cell lines were investigated. Our data confirmed that all the tested extract has cytotoxic activity against HepG2 cell lines up to some extent. Previous studies also reported that *Avicenina marina* (Eldohaji et al., 2021; Yang et al., 2018), *Phragmites australis* (El-Borady et al., 2021; Hosny et al., 2021; Oladipo et al., 2020; Petropoulos et al., 2018), *Moringa oleifera* (Barhoi et al., 2021; Mumtaz et al., 2021) and their refined constituents possess cytotoxic behavior against cancer cells. Possible modes of action for cytotoxic behavior could be; cell shrinkage, compacting cytoplasm, chromatin condensation, pyknosis, and DNA fragmentation (Obeng, 2020). In this study, *Moringa oleifera* showed maximum cytotoxic potential as compared to the rest of the treatments. We found that *Moringa oleifera* contains maximum phenolic contents as compared to extracts of *Avicenina marina* and *Phragmites australis,* which might be the major contributor to its anticancer activities. The findings of Mumtaz et al. (2021) also support our point that *Moringa oleifera* based phenolic contents (i.e., gallic acid, p-coumaric acid, quercetin, and 4-hydroxy 3- methoxy cinnamic acid) depict cytotoxic behavior and potential against cancer cell cells.

Fig. 12. Pearson's correlation among the total phenolic contents, antioxidant activities, and cell viability. Where, MTT: Cell viability (%) by MTT assay against HepG2 cancer cell lines; TPC: Total phenolic contents; FRAP: Antioxidant activity by ferric reducing antioxidant power assay; TAO: Total antioxidant activity by phosphomolybdenum method; DPPH: Antioxidant activity by 2,2-diphenyl picryl hydrazyl ($p \leq 0.05$).

5. Conclusion

In this study, we compare antimicrobial, antioxidant, and cytotoxic activities of *A. marina*, *P. australis,* and *M. oleifera* based ethanolic plant extracts. The activities of plant extracts vary from species to species, plant habitat, and plant parts. As phenols are the major constituents of plant extracts, the antioxidant activities were significantly positively correlated with phenolic contents of respective ethanolic plant extracts. *Moringa oleifera* based extract (i.e., 6E) was found more active against HepG2 cell lines comparatively, which was the indicating that phenolic ingredients playing a major role in cytotoxic activities because 6E contains maximum phenolic contents comparatively. As a future thrust, the studies should be conducted for quantification and purification to figure

out complete chemical characterization and active ingredients present in plant extracts that were used in this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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