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Indian Olive, *Elaeocarpus floribundus* Fruit: Perspective to the Antioxidative Capacity and Antibacterial Activity

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Abstract

The olive fruits are famous for medicinal property possessing an array of bioactive phytochemicals. The current study ascertains the antioxidative capacity and antibacterial activity of ethanolic and aqueous extracts of Indian olive, *Elaeocarpus floribundus* fruit extracts. The antibacterial activity of ethanolic olive seed (OSE) and mesocarp-epicarp extracts (OMeE) and aqueous olive seed (AqOSE) and mesocarp-epicarp extracts (AqOMeE) was determined against gram-positive (*Bacillus cereus, Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Proteus vulgaris*) clinical bacteria, by agar-well diffusion method. The antibiotic sensitivity of the bacterial isolates was determined by disc diffusion. The bioactive components, such as TFC (total flavonoids content), TTC (total tannins content) and TPC (total phenolics content) of the olive fruit test extracts were measured spectrophotometrically, while their antioxidant activity were assessed in DPPH system, and the IC_{50} values were determined. The top zone diameter of inhibition (ZDI; 22 mm) was recorded against *S. aureus*, due to the action of OMeE. The highest TFC, TTC and TPC, as found in the OSE, were 2.82 ± 0.11 mg/g, 2.02 ± 0.17 mg/g and 8.60 ± 0.52 mg/g, respectively, and the OSE antioxidative activity ranged 43.473 - 92.42%, with IC_{50} values 1.12 - 6.46 mg/ml. The *E. floribundus* fruits available from local niches might be utilised as a rich source of plant-based natural antioxidants, and as the non-antibiotic biotherapeutics against bacterial infection to humans.

Keywords: *Olive Fruit; Phytochemicals; Antioxidative Capacity; IC50 Value; Bacterial Pathogens; Non-Antibiotic Antibacterials*

Abbreviations

ANOVA: Analysis of Variance; ATCC: American Type Culture Collection; AqOMeE: Aqueous Olive Mesocarp-Epicarp Extract; AqOSE: Aqueous Olive Seed Extract; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; GAE: Gallic Acid Equivalent; IC_{50}: Median Inhibitory Concentration; MRSA: Methicillin Resistant *Staphylococcus aureus*; MTCC: Microbial Type Culture Collection; OD: Optical Density; OMeE: Ethanolic Olive Mesocarp-Epicarp Extract; OSE: Ethanolic Olive Seed Extract; QD: Quercetin Dihydrate; SD: Standard Deviation; TFC: Total Flavonoids Content; TPC: Total Phenolics Content: TTC: Total Tannins Content; ZDI: Zone Diameter of Inhibition

Introduction

Various human pathogenic bacteria, having the capacity to cause life-threatening diseases in humans, develop multidrug resistance among them, due to high antibiotics exposure [1] and dose discontinuation of antibiotics [2], thereby causing treatment failures [3]. To combat the problem related to bacterial antibiotic resistance, alternative drugs remain the foremost choice, and thus replacement of the conventional medicines are required, however, there are meagre arrangements available for the new antibiotics as per the need. Considering the fact, scientists are working continuously in order to search new sources of antimicrobial agents that are naturally obtained, such as honey [4], probiotic [5], plants [6,7], and so on. On the other hand, in order to ameliorate the problem of oxidative stresses and metabolic disorders, the scientists around the world are devoted to search the natural sources of antioxidative agents [8]. Several native plants pos-

sessed many antioxidant components, such as phenolics, flavonoids, tannins, alkaloids and terpenoids, as has been reported by earlier authors [9-12]. The olive plants, *Elaeocarpus floribundus*, belong to the family Elaeocarpaceae, and are found mainly in the lowland hills in various countries, including India [13]. The *E. floribundus*, is a well-known plant possessing the capacity to show various biological activities: antibacterial [14], antioxidants [13,14], anti-aging [15], as well as the medicinal property, such as antitumor or anticancer activities [13,15]. The bark and leaf of *E. floribundus* were reported to use as mouth wash, while the fruits possessed antiseptic property [16]. The *E. floribundus* fruit parts, showed reducing and stabilizing activities in the biosynthesis of silver nanoparticles having growth inhibition activity against gram-positive and gram-negative bacteria [17]. The phytochemicals from aqueous and ethanolic olive seed and mesocarp-epicarp extracts assessed have been reported [17-19], and the antibacterial activity was determined against clinical MRSA isolates as well as food-borne bacterial isolates [19,18]. Herein, various extracts of *E. floribundus* fruit parts have been prepared for quantitative estimation of phytochemicals (total flavonoids, tannins and phenolics), and determination of antioxidative property and antibacterial activity against clinical bacterial isolates.

Materials and Methods

Bacterial strains

The clinical isolates of gram-positive: *Bacillus cereus* and *Staphylococcus aureus*, and gram-negative: *Escherichia coli, Proteus vulgaris*, bacteria were used in the current study, while the standard strains included were *Listeria monocytogenes* MTCC 657 and *Pseudomonas aeruginosa* ATCC 27813.

Olive fruit extract preparation

The seed and mesocarp-epicarp of mature fruits from olive plant, *E. floribundus*, were collected from wild plants at Hemtabad block (Latitude: 25° 40' 59.99" N and Longitude: 88° 12' 60.00" E) [20] of Uttar Dinajpur district, West Bengal, India. The extracts were prepared as mentioned elsewhere [18], using 25g dried materials for ethanolic and 10g for aqueous extracts. The prepared extracts, containing 125 µg/µl, for OSE and OMeE, and 166 µg/µl, for AqOSE and AqOMeE, were stored at 4^0C, for further studies.

Antibiotic susceptibility test

The clinical bacterial isolates were tested against antibiotics (Hi-Media, India): ampicillin (AMP; 10-µg/disc), cefotaxime (CTX; 30-µg/disc), chloramphenicol (C; 10-µg/disc), gentamycin (GEN; 30-µg/disc), imipenem (IPM; 10-µg/disc), meropenem (MRP; 10-µg/disc), methicillin (MET; 5-µg/disc), piperacillin (PI; 100-µg/disc), piperacillin/tazobactam (PIT; 100/10-µg/disc), and vancomycin (V; 30-µg/disc), following disc diffusion method [21], as per the recommendation of the CLSI guidelines [22].

Antibacterial activity of olive fruit extract

The agar-well method was used for the determination of antibacterial activity of olive fruits test extracts, according to Tagg and McGiven [23]. Test bacterial broth culture swabbed on the nutrient agar plate superficially, and wells were equipped of about 6 mm diameter and extracts of 50 µl (equivalent to 6.25 mg/well for OSE and OMeE, and 8.3 mg/well for AqOSE and AqOMeE) were filled in each well [24]. ZDIs found around the wells after 24h incubation (at 37°C), and were measured. Interpretation of ZDIs confirmed as highly active with ZDIs ≥ 15 mm, less active (ZDIs: ≤ 10 mm) or moderately active (11 - 14 mm) [25].

Quantitative estimation of bioactive phytochemicals

Estimation of flavonoids

The TFC of olive fruit extracts (OSE, OMeE, AqOSE and AqOMeE) were measured following aluminum chloride colorimetric method [26,27], with slight modifications. Briefly, in 200 µl plant part extract, 10% aluminum chloride solution (50 µl) and 50 µl potassium acetate (1M) were added, and the mixture volume was adjusted to 2000 µl, with the addition of double distilled water. After 30 min incubation (at room temperature), in dark place, with the development of blue colour, the OD of the sample was measured spectrophotometrically at

415 nm. The quercetin dihydrate was used to construct the standard curve, and the TFC are recorded in terms of QD equivalent (mg/g of the extract). The tests were performed thrice and the results were documented as mean ± SD.

Estimation of tannins

The TTC of the olive fruit test extracts were determined according to Tamilselvi., *et al* [28]. Briefly, to 100 µl of the extract, 500 µl Folin-Ciocalteau reagent and 1000 µl sodium carbonate solution (35%) were added, the volume was adjusted to 2000 µl, with the addition of double distilled water, and incubated in dark place for 30 min, to allow the development of blue colouration. The OD of the sample was measured spectrophotometrically at 725 nm; GA was used for control standardization [4]. Total tannin values were stated in terms of GA equivalent (mg/gm of the extract). Tests were executed thrice and the values were recorded as mean ± SD.

Estimation of phenolics

The TPC olive fruit extracts were measured according to Kaur and Kapoor [29], with some modification. In 100 µl plant, 1500 µl distilled water, 250 µl folin Ciocalteau reagent, and 1000 µl sodium carbonate solution (20%) were added, and mixed properly. After 30 minutes incubation in dark place, blue colour was developed, and the OD of the sample was measured spectrophotometrically at 650 nm. The GA was used for control standardization [4]. The TPC values were expressed in terms of GAE (mg/gm of extract). The tests were executed thrice and outcomes were recorded as mean ± SD.

Antioxidative activity

The antioxidative activity of both ethanolic and aqueous olive fruit extracts were determined in DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay system. The reaction mixture prepared with different concentration of test extracts: 0.006, 0.008, 0.012, 0.016, 0.020 and 0.024 ml (equivalent to 0.75, 1, 1.5, 2, 2.5 and 3 mg, respectively) for OSE and OMeE, and 0.020, 0.024, 0.028, 0.032, 0.036 and 0.040 ml (equivalent to 3.32, 3.98, 4.65, 5.31, 5.98 and 6.64 mg, respectively), for AqOSE and AqOMeE, and the reaction mixture (3 ml) was adjusted by 0.1 mM DPPH ethanolic reagent. After incubation for 15 min, at room temperature, the OD of the reaction mixture was taken using UV-Vis spectrophotometer, at 516 nm [27]. The control was prepared as above without the test extracts, and the baseline adjustment was done using ethanol. The radical scavenging activity of the extracts, which was stated as the inhibition percentage, was considered with the formula [30]: % inhibition= [1-(A/B)] × 100, A: sample OD, B: control OD; and the IC50 values for each samples were calculated as mentioned earlier [30].

Statistical analysis

The records were expressed as the mean ± SD and assessed by one-way ANOVA (analysis of variance) tests by MS Excel 2010 software; evaluation of antibacterial activity in terms of ZDIs was assessed using the t-test, and the p-value of < 0.05 was considered significant.

Results and Discussion

The antibiotic susceptibility test results are presented in table 1. All the bacteria tested had sensitivity to GEN (ZDIs ranged: 22 - 34 mm), while the isolates were resistant to PI, except *B. cereus*, which had moderate sensitivity to this antibiotic agent. The currently tested pathogenic bacteria possess the capacity to cause several kinds of illnesses in humans [31-34]. Moreover, the human pathogenic bacteria showed resistance to multiple antibiotics [3,35]. The test antibiotics, such as AMP, CTX, C, IPM, MRP, MET, PI, PIT and VA, in the present study, had mixed activity against the bacterial isolates, while *E. coli* and *Pr. vulgaris* showed resistance to 8 antibiotics (AMP, CTX, IPM, MRP, MET, PI, PIT and VA), and *S. aureus* (n = 2) to 5 antibiotics (AMP, C, IPM, MET and VA). Therefore, in order to prevent the infection with multidrug resistant pathogenic bacteria, proper selection of antibiotic is required through antibiotic susceptibility testing. However, the choice of antibiotics is limited [1].

Antibiotic	ZDI (mm) for bacterial isolates						
	BC	SA1	SA2	EC	PV	LM	PA
AMP	21	21	21	6	6	24	6
CTX	22	25	19	6	6	22	23
C	24	30	24	25	16	27	10
GEN	27	34	30	23	22	26	27
IPM	29	42	28	16	13	32	16
MRP	30	30	30	14	15	32	30
MET	14	22	10	6	6	10	6
PI	20	11	16	6	6	6	16
PIT	28	23	20	13	6	24	25
VA	22	19	18	8	9	19	6

Table 1: *Antibiotic susceptibility test results for clinical (BC: Bacillus Cereus; SA1: Staphylococcus aureus 1; SA2: Staphylococcus aureus 2; EC: Escherichia coli; PV: Proteus vulgaris) and standard (LM: Listeria monocytogenes MTCC 657; PA: Pseudomonas aeruginosa ATCC 27813) bacteria.*

AMP: Ampicillin; CTX: Cefotaxime; C: Chloramphenicol; GEN: Gentamicin; IPM: Imipenem; MRP: Meropenem; MET: Methicillin; PI: Piperacillin; PIT: Piperacillin/Tazobactam; VA: Vancomycin; ZDI: Zone Diameter of Inhibition.

Therefore, the antibacterial activity of *E. floribundus* fruit extracts (OSE, OMeE, AqOSE and AqOMeE) was determined, following agar-well diffusion (Figure 1); ZDI values of the test bacteria are represented in table 2. For the standard strains (*L. monocytogenes* MTCC 657 and *P. aeruginosa* ATCC 27813) the *E. floribundus* fruit extracts had ZDIs 6 - 18 mm. Zaman [14] reported the antibacterial efficacy of *E. floribundus* leaf extracts against a number of *S. aureus*, for which the ZDIs ranged 10 - 22 mm; the author also confirmed the effective susceptibility of ethanolic *E. floribundus* leaf extract to *E. coli* (ZDI: 16 mm) and *P. aeruginosa* (ZDI: 18 mm). In the current study, the OMeE activity was high against *S. aureus* 2 isolate (ZDI: 22 mm), compared to that of the other, *S. aureus* 1 isolate (ZDI: 14 mm). The earlier studies reported olive fruit extracts' antibacterial activity against food-borne bacteria (ZDIs: 15 - 28 mm) [18], and MRSA isolates (ZDIs: 15 - 18 mm), too [19]. A different species of the genus *Elaeocarpus*: *E. ganitrus,* had extensive antibacterial action against *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa* and *Klebsiella pneumonia*, following agar-well diffusion [36]. In the instant study, the bacteria showed susceptibility to OMeE and AqOMeE, with mean ZDI of 16.57 ± 3.64 mm (range: 12 - 22 mm) and 13.86 ± 2.27 mm (range: 10 - 16 mm), respectively; the AqOSE had no activity, while the OSE had mean ZDI of 8.43 ± 1.51 mm (range: 6 - 10 mm) against test bacteria. Therefore, the antibacterial activity of mesocarp-epicarp extracts (OMeE and AqOMeE), compared to that of the seed extracts (OSE and AqOSE), was higher, and a significance difference was observed between the two (*p* value: < 0.05).

Source	Bacteria	ZDI (mm)			
		6.25 mg/well		8.3 mg/well	
		OSE$^{\alpha}$	OMeE$^{\alpha}$	AqOSE$^{\beta}$	AqOMeE$^{\beta}$
Clinical bacteria	*B. cereus*	7	19	6	15
	S. aureus1	9	14	6	10
	S. aureus2	10	22	6	15
	E. coli	6	12	6	13
	Pr. Vulgaris	8	13	6	16
Standard strains	*L. monocytogenes* MTCC 657	9	18	6	16
	P. aeruginosa ATCC 27813	10	18	6	12
Statistical value					
Mean value		8.43	16.57	6	13.86
SD		1.51	3.64	0	2.27
p value		0.0003$^{\alpha}$		< 0.000$^{3}\beta$	

Table 2: *Zone diameter of inhibition (ZDI) values from the action of olive fruit parts ethanolic and aqueous extracts against the test bacterial isolates.*

OSE: Ethanolic Olive Seed Extract; OMeE: Ethanolic Olive Mesocarp-Epicarp Extract; AqOSE: Aqueous Olive Seed Extract; AqOMeE: Aqueous Olive Mesocarp-Epicarp Extract; SD: Standard Deviation; ZDI: Zone Diameter of Inhibition.

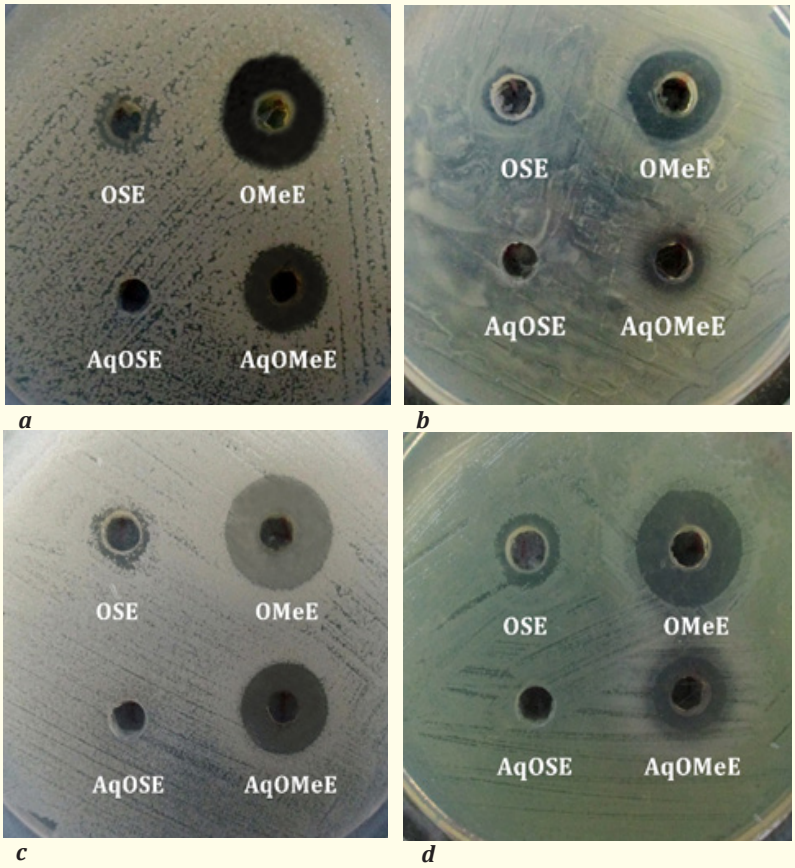

Figure 1: *Agar-well diffusion method demonstrating the antibacterial activity. A: B. cereus; B: S. aureus 1; C: L. monocytogenes MTCC 657; D: P. aeruginosa ATCC 27813. OSE: Ethanolic Olive Seed Extract; OMeE: Ethanolic Olive Mesocarp-Epicarp Extract; AqOSE: Aqueous Olive Seed Extract; AqOMeE: Aqueous Olive Mesocarp-Epicarp Extract.*

The phenolics from olive possess the main antioxidants that act through various mechanisms [37,38]. The other polyphenols, such as flavonoids and tannins, also display antioxidant properties [39]. The levels of TFC, TTC and TPC as estimated, following the gallic acid standard curve and the quercetin dehydrate standard curve (Figure 2), in OSE, OMeE, AqOSE and AqOMeE, are represented in figure 3. The seed ethanolic extract contained maximum amount of bioactive compounds (TFC: 2.82 ± 0.11, TTC: 2.02 ± 0.17, and TPC: 8.6 ± 0.52), mesocarp-epicarp aqueous extract showed good values (TFC: 0.69 ± 0.016, TTC: 0.95 ± 0.02 and TPC: 5.21 ± 0.16); the OSE had highest values of TFC, TTC and TPC. As has been reported earlier [13], the TPC of methanolic *E. floribundus* leaf and stem bark extracts were 503.08 ± 16.71 mg/g GAE and 161.5 ± 24.81 mg/g GAE, respectively, and the antioxidant activity, in terms of IC_{50}, range from 7.36 ± 0.01 µg/ml to 9.37 ± 0.06 µg/ml. The ethanolic, chloroform and petroleum ether extracts of *E. floribundus* had IC_{50} values 45.23, 79.14 and 60.18 µg/ml, respectively, in DPPH system [14]. The antioxidant activities of *E. floribundus* (ethanolic and aqueous) extracts, in DPPH system, are represented in figure 4. The highest concentrations of ethanolic and aqueous test extracts used were 3 mg/ml and 6.64 mg/ml, respectively, at which the top scavenging activities were noted as 92.42%, for OSE, and 61.26%, for AqOMeE, while the lowest IC_{50} was recorded as 1.12 mg/ml, for the OSE, and highest IC_{50} was as 6.46 mg/ml, for AqOSE. Hence, the contents of phytochemicals, such as TFC, TTC and TPC in *E. floribundus* fruit extracts, are proportional to the antioxidant capacity of the extracts. It has been reported that the presence of phenolic compounds might be deviated due to some factors, such as, the extraction time and temperature, reflecting the variation in the antioxidative activities [40].

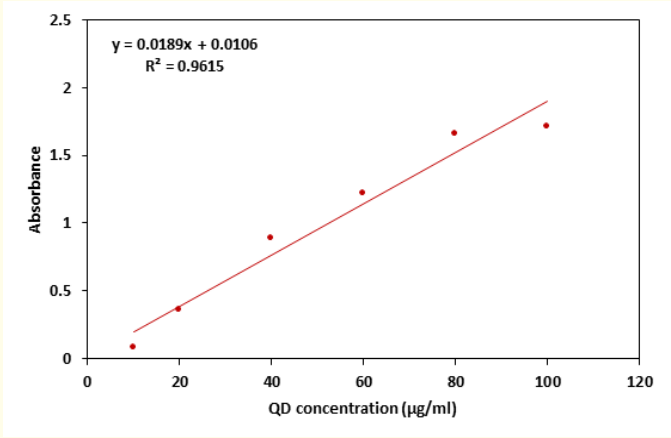

Figure 2: *The quercetin dihydrate standard curve for the determination of total flavonoids content in olive fruit parts extracts.*

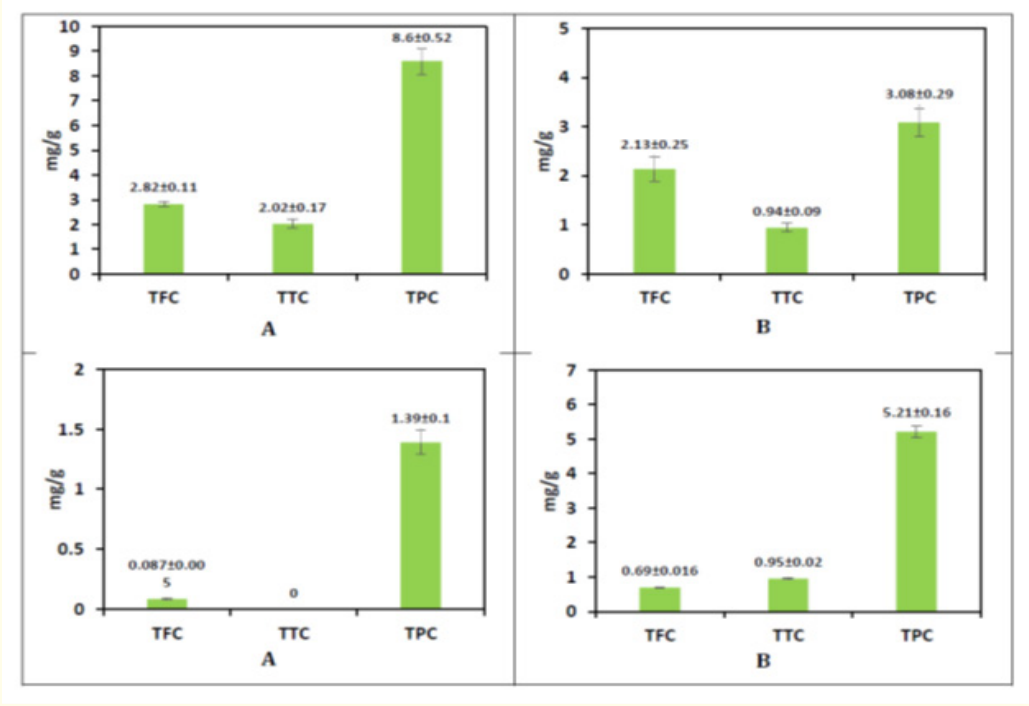

Figure 3: *The TFC, TTC and TPC E. floribundus fruit parts extracts. A: OSE; B: OMeE; C: AqOSE; D: AqOMeE; TFC: Total Flavonoids Content; TTC: Total Tannins Content; TPC: Total Phenolics Content; OSE: Ethanolic Olive Seed Extracts; OMeE: Ethanolic Olive Mesocarp-Epicarp Extracts; AqOSE: Aqueous Olive Seed Extract; AqOMeE: Aqueous Olive Mesocarp-Epicarp Extract.*

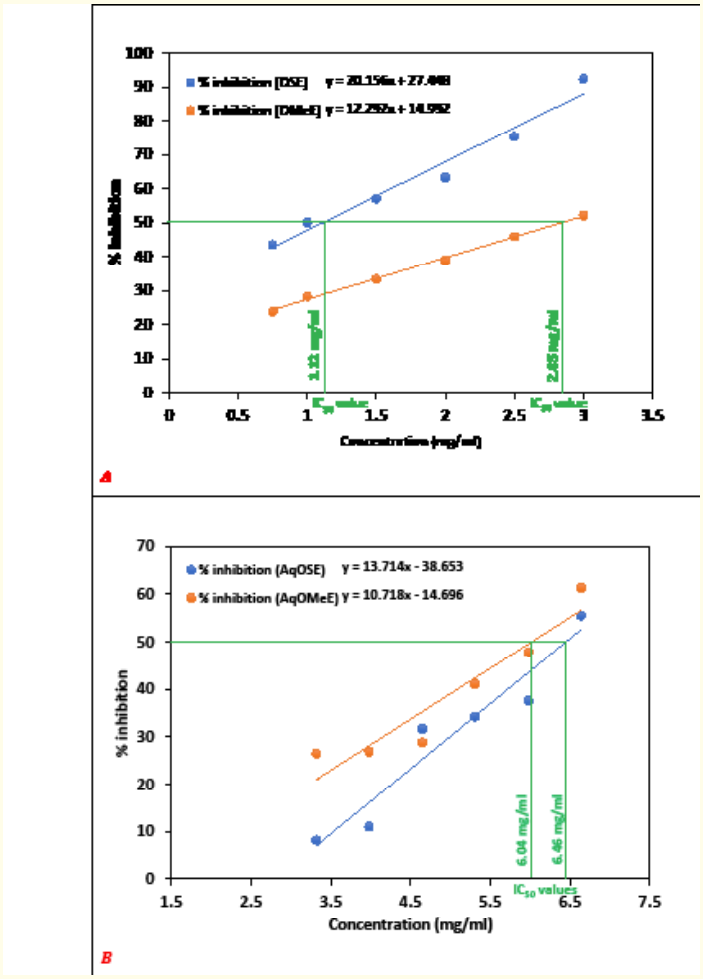

Figure 4: *Antioxidant activity of E. floribundus fruit parts extracts in DPPH system. A: Ethanolic Extracts (OSE and OMeE); B: Aqueous Extracts (AqOSE and AqOMeE).*

Conclusion

The *E. floribundus* fruits from locally available niches displayed broad spectrum of antibacterial activity, and possessed good antioxidative capacity. Therefore, the *E. floribundus* fruits might consumed as a source of natural antioxidants, and can be utilised as a cost effective phytotherapeutics against MDR bacterial infection. Regarding the issues, however, more studies are required in determining the bioactive constituents of *E. floribundus* fruit, responsible for antibacterial and antioxidative capacities, with minimum effective doses.

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Conflicts of Interest

The authors declare no conflict of interest.

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