In vitro antioxidant activity of haloalkaliphilic fungal extracts from lake Magadi, Kenya

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https://doi.org/10.51867/scimundi.5.2.14

ABSTRACT

The wide-ranging saline-alkaline ecological setting is steadily acquiring appreciation as a rich source harbouring a repertoire of extremophilic fungal diversity exerting exclusive biological activities ranging from anti-inflammatory, antipyretic, analgesic among other varied medicinal capacities. However, studies characterizing biochemical functionalities from structurally unique haloalkaliphilic fungal biota remain scanty and undocumented. Importantly, saline emitting hot-springs situated in Rift valley soda lakes are gaining recognition as natural reservoirs with enormous fungal microbial community bearing potential for antioxidation capacity. Therefore, we conducted a cross-sectional laboratory based experimental study through random sampling aimed at characterizing *in vitro* antioxidant activity from haloalkaliphilic fungal strains of Lake Magadi in Kenya. Sample types comprising wet sediments, soils and surface water were cultured in sabouraud's dextrose agar (SDA), potato dextrose agar (PDA) and malt extract agar (MEA) plates at temperatures of 25^0c and 41^0c respectively, for 1-3 weeks. Resulting pure isolates underwent molecular identification. PCR proceeded using ITS-1 & 4 universal primers followed by Sanger sequencing. NCBI's nBLAST supported molecular identification with ≥90% identity cut-off values. Fermentation and extracts production progressed for 28 days at 25^0c accompanied by lyophilisation. Yielded freeze-dried extracts were profiled for antioxidant activity through hydroxyl, superoxide, DPPH, hydrogen peroxide, FRAP and lipid peroxidation inhibition assays. Extracts' total phenolics and flavonoids content were also estimated. IC_{50} was tabulated based on dose-response curves against standards through linear regression. One-way ANOVA compared means across treatments and Tukey's post hoc used for pairwise group comparisons. Statistical significance was considered at $P \leq 0.05$. Genera Cladosporium exhibited dominance (n=4) among sampled fungal biota. Samples P1, P6, P9 and P5 extracts exhibited maximal scavenging activity at higher concentrations against hydroxyl (76.53% ± 1.27), superoxide (78.90% ± 1.29), H_20_2 (76.19% ± 0.40) and DPPH (80.19% ± 0.94) radicals, respectively. Ferric reductive (0.583 ± 0.005) and lipid peroxidation inhibitive (80.95% ± 1.07) activities for isolate P5 was statistically higher relative to other profiled extracts. Radical scavenging capacity of respective antioxidant standards was substantially higher against assayed extracts. Profound IC_{50} scavenging effect occurred at extract concentrations between 2.5 - 3.5 mg/ml. P7 extracts revealed peak total phenolic content of 3.61 ± 0.05 mg gallic acid equivalents/mg crude extract at 4mg/ml, while P6 expressed comparable total flavonoid content of 3.32 ± 0.04 mg quercetin equivalents/mg crude extract. Overall, fungi extracts showcased free radicals scavenging ability against reactive species in assorted antioxidant assays. Besides safety profile validation, our extracts demonstrate applicability for antioxidative potential that may further be discerned via comparative *in vivo* and ex vivo murine experimentation models.

Keywords: Antioxidant, Extremophiles, Free Radicals, Haloalkaliphilic, Hot-Springs, *In vitro*, Saline-Alkaline

I. INTRODUCTION

Oxidative stress signifies a disproportion in the systemic ability to generate free radicals compared to adaptive biological mechanisms to counteract reactive intermediates by antioxidant action (Jiang *et al.*, 2021). Harmful radicals' build-up including reactive oxygen species (ROS) markedly disrupts homeostatic redox cell signaling pathways and induces molecular damage with irreversible tissue injury (Vona *et al.*, 2019). The biochemical process of oxidative stress is regulated by enzymatic and non-enzymatic reactions that mitigate generation and accumulation of by-products of metabolism (He *et al.*, 2017). Distinct enzymatic antioxidants constitute superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and ascorbate peroxidase (APx) whereas their non-enzymatic counterparts predominantly encompass non-organic compounds including isocoumarins, phenolic acids, bioflavonoids, carotenoids and vitamins (Rani *et al.*, 2021; Averill-Bates, 2023).

Notably, SOD existence in aerobic cells and extracellular fluid modulates oxidative stress by degrading hydrogen peroxide (H_2O_2) into less reactive products while averting hydroxyl (OH) radicals formation (Younus, 2018). The GPx enzyme exhibiting abundance in the kidney, liver and erythrocytes curbs oxidative damage by inhibiting lipid peroxides and singlet oxygen scavenging (Csiszar *et al.*, 2016). Conversely, GR seldom scavenges free radicals directly but upregulates radical scavenging action of other antioxidant compounds (Couto *et al.*, 2016). The non-enzymatic bioactive products specifically counteract free radical molecules generation through donation of hydrogen (H^+) atoms from their structures thus disabling apparent radical chain reactions and associated oxidative effects (Rani *et al.*, 2021).

Pronounced biochemical assays adopted in profiling antioxidant capacity via *in vitro* experimentation have formerly been documented and constitutes SOD, H_20_2, GPx, OH, FRAP (ferric reducing antioxidant power), TAC (total antioxidant capacity), nitric oxide (NO), *1,1-diphenyl-2-picril hydrazyl* (DPPH), *2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid* (ABTS), lipid peroxidation inhibition and phosphomolybdenum radical scavenging assays, respectively (Kotha *et al.*, 2022). These antioxidative bioassays exert their chemical scavenging effects through free radical scavenging activity from amongst peroxyl, superoxide, hydroxyl, singlet oxygen and nitric oxide radicals (Ahmadinejad *et al.*, 2017). Notable discrete modes of activity rely on blockade of free radical auto-oxidation reactions, molecular oxygen quenching, hydro-peroxides bioconversion including stabilization and metals chelation action (Vladkova *et al.*, 2022).

Haloalkaliphilic fungi comprise an ecologically defined class of organisms showcasing adaptive physiological attributes that confer resistance to abiotic stressors (Moubasher *et al.*, 2018). They yield considerable amounts of extremozymes and extremolytes that further propagates their tolerability (Dendouga and Belhamra, 2022). The extensive saline deposits that release into alkaline lagoons among East African Rift valley soda lakes including Lakes' Magadi, Bogoria, Natron and Sonachi enables existence of extremophilic microbial species with marked halotolerant and alkaliphilic characteristics (Ndwiga *et al.*, 2015; Salano *et al.*, 2018; Orwa *et al.*, 2020). The capacity for natural products synthesis from haloalkaliphilic fungal biota is reliant on wide-ranging considerations inclusive of fungal species and strain, ecological growth parameters as well as fermentation, extraction and purification approaches adopted (Alvarez-Gomez *et al.*, 2016; Vitale *et al.*, 2020).

Fermentation extracts from haloalkaliphilic fungi display an assortment of biological effects including antioxidant activity (Saravanakumar *et al.*, 2020). In particular, polyphenolic derivatives especially phenolic acids and flavonoids are formerly revealed to possess proton radical-scavenging ability that confers protection of cellular structures from oxidative stress and lipid peroxidative damage (Caparica *et al.*, 2020). Flavonoids such as quercetin, naringenin, and kaempferol as well as phenolic acids including gallic acid, p-coumaric acid, ferulic acid, cinnamic acid and p-hydroxybenzoic acid from vast extremophilic fungi modulate systemic oxidative states by scavenging peroxyl radicals, suppressing molecular oxygen and inhibiting autoxidation reactions (Rani *et al.*, 2021; Vladkova *et al.*, 2022).

Moreover, they exhibit antitumorigenic potential through induction of autophagy-associated cell death alongside ROS dependent premature senescence and apoptosis (Hasima and Ozpolat, 2014; Mishra *et al.*, 2020). Yet still, methyl (*3S)-3-(2,3-dihydroxyphenyloxy*) butanoate, a fermentation product of *Cladosporium* sp. OUCMDZ-302 demonstrates scavenging action against DPPH radicals with profound IC_{50} value of 2.65 µM (Wang *et al.*, 2018). Likewise, ergosterol peroxide isolated from fermented extracts of *Aspergillus fumigatus, Cladosporium cladosporioides and Fusarium equiseti* reveals antioxidant bioactivity by retarding lipid peroxidation of adipocytic tissues (Jeong *et al.*, 2020). The present study purposed to profile for *in vitro* antioxidant activity in haloalkaliphilic fungal extracts from Lake Magadi in Kenya that aims to form a steady basis for supplementary exploration of potent biologically active compounds with previously uncharacterised medicinal capacities.

1.1 Statement of the Problem

The pursuit for naturally sourced antioxidants has intensified in recent times, owing to adverse effects attributed with synthetic antioxidants presently available including propyl gallate (PG), butylated hydroxytoluene (BHT),

butylated hydroxyanisole (BHA), octyl gallate, resveratrol and phycocyanin (Williams and Iatropoulos, 2017; Kobets *et al.,* 2022). In particular, BHA, BHT and PG reveal extensive applicability by food and pharmaceutical industries in retarding short-chain fatty acid oxidation (Kim *et al.,* 2016). However, their prolonged use hastens tissue accumulation primarily in the liver resulting in deranged metabolic functionalities (Liu and Mabury, 2020). Intake exceeding dietary allowable limits exacerbates cellular injury through premature senescence effects and induced oxidative stress that heightens carcinogenesis risk (Xu *et al.,* 2021). Contrastingly, the bulk of their natural counterparts exhibit considerable quantities of polyphenolics, carotenoids and vitamins that efficiently counteract oxidative episodes (Lourenco *et al.,* 2019). Interestingly, fungal biota from halotolerant and alkaliphilic biosystems account for a rich but under-exploited source of naturally derived products with potential for antioxidant activity (Vladkova *et al.,* 2022). As such, screening for *in vitro* antioxidative action may reveal their utility as alternative sources for synthesizing organic sourced oxidative stress modulating agents. To the best of our knowledge, no preceding Kenyan study has demonstrated the aforementioned feature among halotolerant and alkaliphilic fungi isolates.

1.2 Research Objective

This study sought to evaluate the *in vitro* antioxidant activity of fungal extracts from Lake Magadi, Kenya.

II. METHODOLOGY

2.1 Study Setting, Design and Sampling

This was a cross-sectional laboratory based experimental study, using simple random sampling in Lake Magadi, Kenya. The 100 square km body represents southern-most Lake in Kenyan Rift valley lying 600m above sea level in catchment of faulted volcanic rocks enriched with saline emitting hot-springs that discharge into alkaline lagoons. Enormous saline deposits enhances thrive of halotolerant fungal biota creating ideal study site (Lanzen *et al.,* 2013). The sampling points surrounded the discharging hot-springs positioned along the North-western and Southern shore-lines where study specimens (sediments, soils and surface water) were randomly obtained.

2.2 Samples Collection, Handling and Processing

Approximately 30 gm of sample types encompassing wet sediments and surrounding soils were collected using pre-sterilized hand scoops into zip-lock bags, while 50 ml surface water was aseptically aspirated via syringes into leak-proof screw capped universal bottles. Transportation progressed under dry ice with overnight storage at -20^0c awaiting processing. I gm each of pooled soil and sediment samples were emulsified in 10 ml distilled water with slight vortexing for 5 min. A 10-fold serial dilution of contents ensued with 100 µl aliquot of highest dilution (10^{-1}) being separately inoculated by spread plate technique into sabouraud's dextrose (SDA), potato dextrose (PDA) and malt extract (MEA) agars. Growth media reconstitution was based on manufacturer's protocol with slight modifications incorporating 3.5% sodium chloride per litre and final pH of 9 via sodium hydroxide (NaOH) pellets that facilitated heightened cultivation of haloalkaliphilic fungal species (Salano *et al,* 2017; Orwa *et al.,* 2020).

23.3 Specimen Isolation and Identification

Culture plates were incubated aerobically in an inverted position at room temperature (RT; 25^0c) and 41^0c for 1 – 3 weeks with consistent evaluation of fungal sporulation and mycelial growth. Pure cultures were compared using published fungal identification keys and reference illustration tables (Hunter and Barnett, 2019; Pitt and Hocking, 2022), before proceeding for molecular identification by Sanger sequencing. The NCBI's nucleotide blast (nBLAST) matched sequences similarity with ≥90% percentage identity values being adopted to represent prospective fungal taxa. The number of recovered isolates was based on varied fungal selective growth characteristics on diverse cultivation media types.

2.4 Fermentation and Production of Extracts

Pure fungal isolates were propagated in glass Erlenmeyer flasks containing 350 ml pre-sterilized sabouraud's dextrose (SDB) and potato dextrose (PDB) broths constituted as per manufacturer's specifications. Broth media constituents facilitating cultivation of yeasts and molds extracts comprised of 20 g/L dextrose, 5g/L peptone, 3g/L yeast for SDB and 4g/L potato starch (potato infusion) and 20g/L dextrose for PDB. Slight fermentation media adjustments incorporated 3.5% sodium chloride per litre and pH levels alteration for sustenance of hypersaline-alkaline provisions (Orwa *et al.,* 2020). Ampicillin-streptomycin (Duchefa Biochemie®, Europe) at concentrations of 100 mg/L inhibited bacterial colonization. Fermentation to yield extracts proceeded at RT on a rotary shaker at 165 rpm for 28 days (Vitale *et al.,* 2020). A flask with pre-sterilized broth media lacking inoculum functioned as negative control.

2.5 Extracts Preparation for *in vitro* Antioxidant Analysis

Approximately 400 mg of freeze-dried extracts were weighed using an analytical balance (Meubon®, Texas, USA) and dissolved in 100 ml distilled water. Resultant contents were uniformly vortexed at low speed for 10 min and filtered via Whatman no. 1 paper (Whatman™, Buckinghamshire, England) and pre-sterilized double-layer muslin cloth. Consequently, the respective extract stock solutions of 4 mg/ml were subjected to 6-fold serial dilution ranges from 4 – 1.5 mg/ml. All experimentation proceeded by triplicate measurements with extracts 50% radical scavenging activity (IC_{50}) also determined.

2.6 Experimentation Assays for Extracts *in vitro* Antioxidant Activity

2.6.1 Hydroxyl (OH) Radical Scavenging Assay

Principle: Assay determines capacity of an extract to inhibit hydroxyl radicals formation by degradation of deoxyribose in the Fenton's reaction (Talvenmaki *et al.,* 2019). *Procedure:* Briefly, a 1 ml reaction mixture was constituted using 0.1 ml [28 mM] deoxyribose, 0.2 ml [1 mM] $FeCL_3$, 0.2 ml of [20 mM] potassium phosphate buffer pH 7.4, 0.1 ml [1 mM] ascorbic acid, 0.1 ml [1.04 mM] EDTA, 0.1 ml [1 mM] H_20_2 and 0.2 ml of sample (extract) or gallic acid (positive control). Contents were incubated for 45 min at room temperature (RT) with subsequent addition of 1 ml each of 1% *w/v* thiobarbituric acid (TBA) and 2.8% trichloroacetic acid (TCA) and further 20 min incubation at 100^0c. Thereafter, absorbances were measured at wavelength (λ) 532 nm via a UV/Vis double beam spectrophotometer (Shimadzu®, model 1601, Kyoto, Japan), with percentage hydroxyl scavenging activity (% HSA) expressed using standardized methods (Pavithra and Vadivukkarasi, 2015).

$$\% \text{ HSA} = \frac{A_C - A_S}{A_C} \text{ X } 100\%$$

Where:

A_C = control absorbance

A_S = extract or standard absorbance

2.6.2 Superoxide (SOD) Radical Scavenging Assay

Principle: Test is based on the ability of extract to neutralize superoxide radicals, thus hindering formation of purple formazan from nitro blue tetrazolium (NBT) reduction (Apak *et al.,* 2022). *Procedure:* Briefly, 3ml reagent mixture comprising 0.1 ml [50 mM] NBT, 0.2 ml [12 mM] EDTA, 1ml [20 µg] riboflavin and 1.7 ml [50 mM] sodium phosphate buffer pH 7.6 was mixed with 1 ml extract and contents illuminated via photometric light source for 10 min at RT. Formazan present in extracts was immediately quantified at λ 562 nm. Absorbance readings were obtained for the positive control (quercetin) and blank solution. Percentage SOD scavenging by NBT reduction was computed as previously described (Zhang *et al.,* 2016).

$$\% \text{ SOD activity} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Abs Control}} \text{ X } 100\%$$

Where:

Control Abs = absorbance of control (blank)

Sample Abs = absorbance of extract or quercetin

2.6.3 DPPH Radical Scavenging Assay

Principle: Extract with antioxidant compound reduces *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical to hydrazine, a stable non-radical molecule. Test solution changes colour from deep violet to yellow at reducing absorbance wavelength of 517 nm (Gulcin and Alwasel, 2023). *Procedure:* Briefly, 19.7 mg DPPH salt was dissolved in 50 ml methanol to form [1 mM] methanolic solution of DPPH. 0.5 ml of reaction mixture was added to 1 ml of extract and contents slightly vortexed before incubation in the dark at RT for 20 min. Absorbance was immediately measured at λ 517 nm with ascorbic acid serving as positive control and mixture of methanol and DPPH radical as negative control. % RSA was expressed using an equation previously described (Kibiti and Afolayan, 2015).

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \text{ X } 100\%$$

Where:

Absorbance control = absorbance of methanol + DPPH radical

Absorbance test = sample extract + methanolic solution of DPPH

Blank solution = sample extract + methanol solution

2.6.4 Hydrogen Peroxide Radical Scavenging Assay

Principle: Test is based on ability of extract to degrade hydrogen peroxide (H_2O_2) with decreasing absorbance at λ230 nm (Malik *et al.*, 2011). *Procedure:* Briefly, 1 ml extract was mixed with 0.6 ml reaction mixture composed of [40 mM] H_2O_2 with phosphate buffer pH 7.4, and contents incubated for 10 min at RT. Absorbance was read at λ 230 nm for respective extracts, control (ascorbic acid) and blank solution. Measurements were conducted in triplicates with percentage H_2O_2 scavenging calculated through the formula:

$$\% \, H_2O_2 \text{ scavenging activity} = \frac{A_1 - A_0}{A_1} \times 100\%$$

Where:

A_1= control absorbance (blank)

A_0 = extract or ascorbic acid absorbance

2.6.5 Ferric Reducing Antioxidant Power (FRAP) assay

Principle: An antioxidant compound reduces ferric to ferrous iron whose colour intensity (purple) is spectrophotometrically quantified and proportionate to extract's ferric reducing power (Moriasi *et al.*, 2020). *Procedure:* Briefly, a uniform mixture of 1.25 ml potassium ferricyanide [30 mM] and 0.5 ml phosphate buffer [200 mM] pH 6.6 was added to 1 ml extract volume and contents gently vortexed for 5 min before incubation at 53^0c for 20 min. Afterwards, 1.25 ml TCA, [600 mM] was added with centrifugation for 10 min at 3000 rpm. 1 ml of supernatant was aliquoted to a clean tube and mixed with 0.5 ml $FeCL_3$ [600 mM] and 0.75 ml distilled water. Absorbance readings of respective extracts, control (ascorbic acid) and blank were determined at λ 700 nm.

2.6.6 Lipid Peroxidation Inhibition Assay

Principle: Malondialdehyde (MDA) reacts with TBA (thiobarbituric acid) to form a pink product assessed spectrophotometrically at λ 532 nm (Khoubnasabjafari *et al.*, 2015). *Procedure:* Briefly, 2 ml homogenate volume of TBA (0.375% *w/v*), TCA (15% *w/v*) and HCL (0.25 N) were mixed with 1 ml extract with final reaction mixture incubated for 15 min at 90^0c. Subsequently, samples were centrifuged for 10 min at 5,000 rpm with aspiration of pink coloured supernatant. Relative yield of MDA was measured at λ 532 nm for extracts and compared against control (ascorbic acid) and blank solution. Tests were conducted thrice with percentage lipid peroxidation inhibition calculated:

$$\% \text{ lipid peroxidation inhibition} = \frac{A_C - A_S}{A_C} \times 100\%$$

Where:

A_C = control absorbance (blank)

A_S = test extract or ascorbic acid absorbance

2.6.7 Total Phenolic Content of Extracts

Principle: Phenolic groups in the test extracts reduces folin-ciocalteu reagent to yield molybdenum- tungsten blue measured spectrophotometrically at λ 765 nm (Kupina *et al.*, 2018). *Procedure:* Briefly, 2ml of [2%] $NaHCo_3$ were mixed with 1 ml extract and incubated for 2 min at RT. Subsequently, 0.2 ml folin-ciocalteu was pipetted into reaction mixture with added dark room incubation for 30 min at RT. Optical densities were measured at 765 nm with gallic acid adopted as standard control and methanol replacing Folin's phenol reagent as blank solution. Total phenolics content was expressed as mg/ml gallic acid equivalents/mg sample extract.

2.6.8 Total Flavonoids Content of Extracts

Principle: Assay evaluates capacity of keto and hydroxyl groups of flavones and flavonols in test extract to form acid-stable complexes with $ALCl_3$, that is measured calorimetrically at λ 420 nm (Shraim *et al.*, 2021). *Procedure:* Briefly, a blended reaction mixture of 0.15 ml [10%] $ALCl_3$), 0.34 ml [30%] ethanol, 0.3 ml extract and 3 ml distilled water were slightly vortexed and incubated at RT for 8 min before addition of 1 ml NaOH and absorbance measured at 510 nm relative to the blank. Quercetin was incorporated as positive control with total flavonoids content expressed as mg/ml quercetin equivalents/mg crude extract.

2.7 Ethical Consideration

Ethical approval was granted by Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMU/COR: 403012 Vol 2(5). Consent was not required for study participation.

2.8 Data Management and Statistical Analysis

Absorbance measurements were organized, cleaned and tabulated in Microsoft Excel spreadsheets (Ms® Office 365). Statistical analysis proceeded using GraphPad Prism v8 software (GraphPad™, California, USA). One-way ANOVA compared means across test groups followed by Tukey's post hoc for pairwise comparisons. Data was expressed as Mean ± Standard Error of Mean ($\bar{x}$ ± SEM) and presented in form of tables. IC_{50} values were inferred graphically by linear regression analysis. Study extracts phenolics and flavonoids content were established following estimation of correlation coefficient (R^2) values. All statistical significance was considered at $P \leq 0.05$.

III. FINDINGS

Nine (9) study isolates exhibited growth in assorted culture media types and proceeded for molecular identification. ITS gene sequence analysis by nBLAST profiled the fungal species into **P1;** *Fusarium incarnatum* isolate F31 **P2;** *Fusarium oxysporum* sp. *lycopersici* strain FO43 **P3;** *Sarocladium* sp. voucher GJB **P4;** *Fusarium equiseti* isolate ATS37 **P5;** *Aspergillus fumigatus* isolate AA **P6;** *Cladosporium cladosporioides* strain JZY1-25 **P7;** *Cladosporium oryzae* isolate ihWWF158 **P8;** *Cladosporium cladosporioides* isolate wb413 **P9;** *Cladosporium parahalotolerans* strain 299N1 (Table 1). Subsequently, yielded fermentation extracts from pure study isolates were subjected to diverse *in vitro* antioxidant analysis.

Table 1

nBLAST Analysis Results for ITS Gene Sequence of Lake Magadi Fungal Isolates

Isolate Code	Accession Number	% Identity	Closest Relative BLAST
P1	KX523892.1	95.7%	*Fusarium incarnatum* isolate F31
P2	KF914438.1	100%	*Fusarium oxysporum* sp. *lycopersici* strain FO43
P3	MH890606.1	99.4%	*Sarocladium* sp. voucher GJB
P4	KY436192.1	91.8%	*Fusarium equiseti* isolate ATS37
P5	MT994683.1	91.8%	*Aspergillus fumigatus* isolate AA
P6	MT786364.1	97.6%	*Cladosporium cladosporioides* strain JZY1-25
P7	MG385089.1	98.9%	*Cladosporium oryzae* isolate ihWWF158
P8	AF455442.1	99.7%	*Cladosporium cladosporioides* isolate wb413
P9	OP237121.1	98.9%	*Cladosporium parahalotolerans* strain 299N1

BLAST; Basic local alignment search tool. nBLAST; Nucleotide BLAST. ITS; Internal transcribed spacer.

3.1.1 *In vitro* Hydroxyl Radical Scavenging Activity of Fungal Extracts

Hydroxyl radical scavenging ability differed significantly in a concentration-dependent manner with highest treatment concentration exhibiting most potent hydroxyl scavenging capacity ($p \leq 0.05$; Table 2). Isolate P1 extracts showed higher radical scavenging percentage (76.53% ± 1.27) among profiled extracts relative to P3 (66.17% ± 0.54), P4 (65.67% ± 0.33), P8 (64.54% ± 1.44) and P9 (62.05% ± 0.82) extracts at 4mg/ml concentration. However, hydroxyl scavenging potency was statistically comparable for isolates P1, P2, P5 and P7 extracts at highest concentration ($p > 0.05$). Nevertheless, the standard (gallic acid) demonstrated greater hydroxyl radical scavenging action compared to extracts ($p \leq 0.05$). Notably, the IC_{50} of gallic acid was substantially lower against all tested extracts (Table 2). Key note: Results expressed as mean ± SEM for triplicate measures (n=3). Means with different lowercase superscript letters along the columns and uppercase superscript letters across the rows are significantly different ($p \leq 0.05$; One-way ANOVA followed by Tukey's post hoc).

Table 2

In vitro Hydroxyl Radical Scavenging Activity of Fungal Extracts

Hydroxyl Radical Scavenging Percentage (%)										
Extract Conc.	**Standard (Gallic Acid)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	44.94±0.99^{eAB}	40.20±0.87^{eB}	46.44±1.20^{eA}	41.07±1.75^{dB}	47.94±0.99^{dA}	44.07±0.76^{eAB}	44.07±0.90^{eAB}	43.95±1.09^{eAB}	30.96±0.33^{fC}	29.21±0.99^{eC}
2mg/ml	59.55±0.99^{dA}	52.93±0.90^{dBC}	52.31±0.87^{dBC}	46.57±0.76^{dD}	53.68±0.76^{cBC}	49.19±0.76^{dCD}	56.43±0.87^{dAB}	55.18±1.41^{dAB}	39.33±1.14^{eE}	34.46±1.35^{dE}
2.5mg/ml	65.42±1.07^{cAB}	66.04±0.87^{cA}	60.80±1.27^{cBC}	55.06±0.65^{cD}	56.31±0.33^{cCD}	56.55±0.78^{cCD}	62.67±1.30^{cAB}	61.05±0.99^{cABC}	46.69±0.82^{dE}	42.95±1.53^{cE}
3mg/ml	71.41±1.23^{bA}	69.79±0.70^{bcA}	64.17±0.90^{bcB}	60.05±0.76^{bB}	60.05±0.66^{bB}	60.55±1.23^{cB}	69.79±1.39^{bA}	64.17±0.87^{bcB}	52.68±0.87^{cC}	54.93±0.70^{bC}
3.5mg/ml	76.16±0.76^{aA}	71.91±1.77^{abAB}	68.41±0.33^{bB}	62.67±0.76^{abC}	62.80±0.76^{abC}	68.79±1.07^{bB}	73.91±1.23^{abA}	68.41±0.98^{bB}	59.05±1.02^{bC}	59.30±0.76^{abC}
4mg/ml	80.52±0.78^{aA}	76.53±1.27^{aAB}	73.03±0.94^{aB}	66.17±0.54^{aC}	65.67±0.33^{aC}	73.41±0.94^{aB}	75.66±1.08^{aB}	73.78±0.78^{aB}	64.54±1.44^{aC}	62.05±0.82^{aC}
IC_{50} mg/ml	1.67±0.02^{D}	1.84±0.03^{CD}	1.76±0.07^{D}	2.17±0.03^{B}	1.68±0.08^{D}	2.03±0.07^{BC}	1.75±0.04^{D}	1.77±0.03^{D}	2.76±0.05^{A}	2.80±0.04^{A}

3.1.2 *In vitro* Superoxide Radical Scavenging Activity of Fungal Extracts

The effectiveness of scavenging superoxide (SOD) radicals differed significantly for fungal extracts, with lower activity observed at lesser extract concentrations ($p \leq 0.05$; Table 3). Conversely, SOD radical scavenging by the standard (quercetin) was considerably higher than assayed extracts especially at 3.5 mg/ml (77.00% ± 1.06) and 4 mg/ml (81.93% ± 1.17; $p \leq 0.05$). Nonetheless, radical scavenging was statistically comparable ($p > 0.05$) for isolates P1 (58.86% ± 1.06), P2 (56.61% ± 0.88) and P9 (66.74% ± 1.17) extracts at similar concentration (4mg/ml). Interestingly, the IC_{50} value of isolate P4 extracts was significantly lower (1.71 ± 0.03 mg/ml) compared to the standard (1.99 ± 0.04 mg/ml; $p \leq 0.05$; Table 3).

Table 3

In vitro Superoxide Radical Scavenging Activity of Fungal Extracts

SOD Radical Scavenging Percentage (%)										
Extract Conc.	**Standard (Quercetin)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	40.08±1.58^{eA}	39.94±0.86^{cA}	24.54±0.49^{eB}	43.88±1.53^{eA}	43.67±0.97^{dA}	42.83±0.53^{eA}	39.31±0.93^{dA}	27.22±1.40^{fB}	40.01±0.61^{eA}	27.07±1.23^{eB}
2mg/ml	51.27±1.38^{dABC}	42.97±0.83^{cE}	29.26±0.69^{dG}	54.15±1.22^{dAB}	56.82±0.95^{cA}	50.84±0.97^{dABCD}	50.56±1.89^{cBCD}	44.94±0.76^{eDE}	48.03±1.59^{dCDE}	36.92±1.20^{dF}
2.5mg/ml	58.65±0.61^{cC}	48.17±1.23^{bD}	35.51±0.43^{cE}	60.48±1.23^{cBC}	68.99±0.65^{bA}	59.49±0.53^{dC}	65.19±1.53^{bcAB}	51.90±1.06^{dD}	66.53±0.86^{cA}	49.93±1.46^{cD}
3mg/ml	69.97±1.04^{cA}	51.41±0.58^{bD}	38.33±0.93^{cE}	67.44±0.99^{bA}	70.61±0.31^{abA}	62.31±0.74^{cB}	70.96±0.67^{bA}	58.93±0.43^{cBC}	71.10±0.73^{bcA}	54.78±1.43^{bcCD}
3.5mg/ml	77.00±1.06^{aA}	56.82±0.93^{aD}	44.73±0.85^{bE}	72.43±0.492^{abB}	72.22±0.39^{aB}	66.03±0.68^{bC}	73.98±0.74^{abAB}	65.40±1.27^{bC}	74.19±0.55^{abAB}	60.20±0.61^{bD}
4mg/ml	81.93±1.17^{aA}	58.86±1.06^{aE}	56.61±0.88^{aE}	75.67±1.13^{aBCD}	73.00±0.24^{aCD}	71.45±0.99^{aDE}	78.90±1.29^{aAB}	72.08±0.90^{aD}	77.43±1.16^{aABC}	66.74±1.17^{aE}
IC_{50} mg/ml	1.99±0.04^{D}	2.76±0.1^{B}	3.72±0.01^{A}	1.8±0.05^{DE}	1.71±0.03^{E}	1.9±0.05^{DE}	1.9±0.04^{DE}	2.29±0.05^{C}	1.93±0.05^{DE}	2.61±0.07^{B}

3.1.3 *In vitro* DPPH Radical Scavenging Activity of Fungal Extracts

In all studied concentrations, DPPH exhibited a concentration-dependent free radical scavenging effect that tallied with extract concentration (Table 4).

Table 4

In vitro DPPH Radical Scavenging Activity of Fungal Extracts

DPPH Radical Scavenging Percentage (%)										
Extract Conc.	**Standard (Ascorbic Acid)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	43.94±0.81^{eA}	25.64±1.85^{eE}	40.04±1.26^{eAB}	36.04±1.27^{eB}	35.31±1.04^{dBC}	34.84±0.41^{eBCD}	30.68±0.86^{fCDE}	29.64±1.09^{fDE}	35.05±0.66^{fBC}	41.39±0.70^{dA}
2mg/ml	58.50±0.83^{dA}	33.65±1.08^{bE}	57.25±0.77^{dA}	48.52±1.24^{dB}	40.25±1.08^{dD}	43.01±0.95^{bCD}	41.19±0.70^{eD}	42.33±1.23^{eCD}	46.96±1.00^{eBC}	50.81±1.62^{cB}
2.5mg/ml	69.74±0.45^{cA}	39.21±0.68^{dE}	61.93±0.86^{dB}	57.05±1.07^{cC}	47.63±1.40^{cD}	55.69±0.41^{dC}	46.75±0.79^{dD}	48.31±0.85^{dC}	54.50±0.50^{dC}	58.35±0.72^{bBC}
3mg/ml	74.57±1.09^{bA}	46.07±0.88^{cF}	67.13±1.05^{cB}	62.51±0.91^{bBcCD}	53.98±0.95^{cE}	65.16±2.82^{aBC}	59.70±0.78^{cCDE}	56.79±1.09^{cDE}	61.67±0.50^{cBCD}	59.91±0.83^{bCDE}
3.5mg/ml	84.50±0.91^{aA}	53.09±1.63^{bE}	72.18±1.16^{bBC}	66.67±1.62^{bCD}	61.78±1.13^{bD}	75.14±2.33^{cB}	65.26±1.69^{bCD}	64.90±1.19^{bD}	68.23±0.96^{bBCD}	64.59±0.50^{aD}
4mg/ml	88.09±0.64^{aA}	66.72±0.81^{aD}	79.72±0.72^{aB}	78.78±1.11^{aB}	75.40±2.39^{aBC}	80.19±0.94^{aB}	77.95±0.81^{aB}	71.30±0.59^{aCD}	76.91±1.35^{aB}	67.55±0.50^{aD}
IC_{50} mg/ml	1.70±0.02^{G}	3.31±0.04^{A}	1.80±0.02^{G}	2.05±0.06^{EF}	2.74±0.08^{B}	2.30±0.03^{CD}	2.58±0.05^{B}	2.52±0.06^{BC}	2.18±0.05^{DE}	1.94±0.03^{FG}

Efficacy of radicals scavenging, as shown in Table 4, was more pronounced for isolates P2 (79.72% ± 0.72) and P5 (80.19% ± 0.94) extracts compared to other isolates predominantly at higher extract concentration ($p \leq 0.05$). The standard (ascorbic acid) displayed markedly higher DPPH radical scavenging capacity relative to study extracts across all tested concentrations ($p \leq 0.05$). Further, the IC_{50} of ascorbic acid was statistically lower (1.70 ± 0.02 mg/ml) compared to tested extracts ($p \leq 0.05$; Table 4).

3.1.4 *In vitro* Hydrogen Peroxide Radical Scavenging Activity of Fungal Extracts

As shown in Table 5, test extracts were markedly efficient in scavenging hydrogen peroxide radicals in a concentration-based manner. In particular, isolate P9 extracts exerted significantly higher H_20_2 scavenging (76.19% ± 0.40) compared to P1 (66.08% ± 0.22), P4 (62.27% ± 1.01) and P8 (62.53% ± 0.94) derived extracts at 4 mg/ml concentration ($p \leq 0.05$; Table 5). Radical scavenging capacity was statistically comparable for the standard (ascorbic acid) and P5, P6, P7, and P9 extracts, respectively ($p > 0.05$). However, IC_{50} value was considerably lower (2.12 ± 0.03 mg/ml) for ascorbic acid against all profiled extracts ($p \leq 0.05$).

Table 5

In vitro Hydrogen Peroxide Radical Scavenging Activity of Fungal Extracts

H_20_2 Radical Scavenging Percentage (%)										
Extract Conc.	**Standard (Ascorbic Acid)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	28.32±1.56^{eAB}	6.59±0.84^{eD}	23.99±1.02^{dB}	17.40±0.91^{eC}	19.12±0.83^{fC}	24.54±0.57^{fB}	8.61±1.26^{eD}	24.76±0.62^{eB}	17.11±0.54^{fC}	30.07±0.47^{fA}
2mg/ml	46.78±0.86^{dA}	23.04±1.25^{dE}	37.91±2.20^{cBC}	25.90±1.72^{dE}	27.84±1.17^{eE}	38.50±0.87^{eBC}	30.00±0.90^{dDE}	43.63±2.29^{dAB}	30.07±0.68^{eDE}	36.45±1.21^{eCD}
2.5mg/ml	58.64±1.12^{cA}	38.35±1.75^{cD}	46.37±1.30^{bC}	46.78±1.24^{cC}	35.13±0.22^{dD}	50.66±0.84^{dBC}	51.06±1.23^{cBC}	54.65±2.24^{cAB}	38.97±1.40^{dD}	50.37±1.01^{dBC}
3mg/ml	67.73±0.48^{bA}	55.57±1.88^{bBC}	57.84±0.55^{aBC}	54.18±2.38^{bC}	46.56±0.22^{cD}	60.66±0.72^{cBC}	59.60±1.67^{bBC}	61.65±0.73^{bAB}	46.56±0.57^{cD}	60.07±1.70^{cBC}
3.5mg/ml	70.66±0.79^{abA}	60.15±0.61^{bCD}	60.11±0.73^{aCD}	61.50±0.49^{aBC}	55.97±0.53^{bE}	68.94±0.37^{bA}	64.69±0.54^{bB}	71.39±0.98^{aA}	57.62±0.89^{bDE}	68.61±0.48^{bA}
4mg/ml	74.54±1.10^{aA}	66.08±0.22^{aB}	63.22±0.67^{aA}	65.35±0.48^{aB}	62.27±1.01^{aB}	72.75±0.58^{aA}	71.98±1.81^{aA}	75.06±0.85^{aA}	62.53±0.94^{aB}	76.19±0.40^{aA}
IC_{50} mg/ml	2.12±0.03^{E}	2.86±0.04^{B}	2.6±0.05^{CD}	2.77±0.04^{BC}	3.18±0.03^{A}	2.47±0.03^{D}	2.52±0.04^{D}	2.27±0.05^{E}	3.12±0.03^{A}	2.55±0.03^{D}

3.1.5 *In vitro* Ferric Reducing Antioxidant Activity of Fungal Extracts

The effectiveness of extracts' ferric reducing ability was characterized by a concentration-related increase in absorbance of reaction mixtures at 700 nm wavelength (Table 6). Notably, isolate P5 extracts exhibited greater ferric reductive activity at higher concentrations, (0.583 ± 0.005), whereas P2 extracts showed least ability to reduce ferric ions across tested extract concentrations ($p \leq 0.05$; Table 6). Nonetheless, ferric reducing capacity was significantly different for the standard (ascorbic acid) and treatment extracts across the different concentrations examined ($p \leq 0.05$; Table 6). The ferric reductive ability of isolate P5 extracts was comparable to that of ascorbic acid at 4mg/ml treatment ($p > 0.05$; Table 6).

Table 6

In vitro Ferric Reducing Antioxidant Activity of Fungal Extracts

FRAP Absorbance (700 nm)										
Extract Conc.	**Standard (Ascorbic Acid)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	0.326±0.021^{dA}	0.226±0.026^{dC}	0.122±0.003^{dD}	0.280±0.009^{cABC}	0.295±0.002^{dAB}	0.260±0.004^{dBC}	0.291±0.013^{dAB}	0.133±0.002^{fD}	0.224±0.015^{cC}	0.148±0.003^{fD}
2mg/ml	0.413±0.006^{cA}	0.255±0.010^{cdE}	0.133±0.003^{dG}	0.316±0.010^{cBC}	0.305±0.002^{dBC}	0.288±0.007^{dCD}	0.326±0.003^{cB}	0.175±0.006^{eF}	0.270±0.006^{bDE}	0.248±0.002^{eE}
2.5mg/ml	0.426±0.007^{cA}	0.262±0.007^{cdD}	0.138±0.004^{cdE}	0.322±0.011^{cC}	0.321±0.005^{cdC}	0.328±0.003^{cBC}	0.357±0.003^{cB}	0.251±0.005^{dD}	0.335±0.004^{cBC}	0.277±0.007^{dD}
3mg/ml	0.452±0.007^{bcA}	0.308±0.011^{bcD}	0.172±0.010^{cE}	0.337±0.015^{bcCD}	0.346±0.002^{cCD}	0.365±0.004^{cBC}	0.396±0.005^{bB}	0.337±0.005^{cCD}	0.365±0.011^{bBC}	0.326±0.003^{cCD}
3.5mg/ml	0.489±0.020^{bA}	0.350±0.002^{bC}	0.239±0.005^{bD}	0.382±0.019^{bBC}	0.384±0.004^{bBC}	0.417±0.018^{bB}	0.421±0.003^{abB}	0.376±0.012^{bBC}	0.415±0.013^{aB}	0.351±0.004^{bC}
4mg/ml	0.622±0.007^{aA}	0.428±0.018^{aC}	0.303±0.013^{aD}	0.515±0.005^{aB}	0.462±0.012^{aC}	0.583±0.005^{aA}	0.441±0.008^{aC}	0.449±0.006^{aC}	0.446±0.005^{aC}	0.450±0.006^{aC}

3.1.6 *In vitro* Lipid Peroxidation Inhibition Activity of Fungal Extracts

The anti-lipid peroxidation activity of study extracts is shown in Table 7. In each extract, maximal peroxidation inhibition effect was observed at the highest treatment concentration. Isolate P8 extracts displayed highest lipid peroxidation inhibition activity (28.87% ± 1.07 – 80.95% ± 1.07) across respective extract concentrations, with statistically comparable values to the standard (ascorbic acid; 27.68% ± 1.36 – 79.17% ± 1.07). Contrastingly, isolate P1 extracts showed least peroxidation inhibitory activity at all assayed concentrations ($p \leq 0.05$; Table 7). Notably, isolates P3, P7, P8, and P9 extracts capacity to inhibit 50% of lipid peroxidation activity (IC_{50}) was markedly higher compared to the standard ($p \leq 0.05$).

Table 7

In vitro Lipid Peroxidation Inhibition Activity of Fungal Extracts

Lipid Peroxidation Inhibition Percentage (%)										
Extract Conc.	**Standard (Ascorbic Acid)**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	27.68±1.36^{fBCD}	13.69±1.07^{fF}	24.40±1.30^{dCDE}	23.51±1.07^{fDE}	31.85±1.81^{dAB}	18.75±1.03^{eEF}	37.50±1.03^{dA}	30.95±1.57^{eABC}	28.87±1.07^{dBCD}	26.79±1.55^{eBCD}
2mg/ml	39.88±1.57^{eCD}	22.32±1.55^{eF}	31.55±0.79^{cDE}	48.51±0.79^{eAB}	43.15±1.81^{cBC}	28.57±1.86^{dEF}	44.35±2.54^{cdABC}	48.81±1.57^{dAB}	52.38±2.32^{dA}	49.11±1.03^{dAB}
2.5mg/ml	52.68±1.36^{dBCDE}	30.65±1.30^{dF}	36.01±1.07^{cF}	53.57±1.03^{dBCD}	47.02±0.30^{cE}	47.32±0.52^{cDE}	52.08±1.66^{cCDE}	56.85±1.30^{cBC}	65.48±2.08^{cA}	58.93±1.36^{cB}
3mg/ml	63.69±1.3^{cB}	38.39±1.55^{cD}	49.11±1.03^{bC}	62.20±1.07^{cB}	60.42±2.84^{bB}	62.20±1.81^{bB}	66.67±2.15^{bAB}	66.37±1.30^{bAB}	72.02±1.30^{bcA}	63.99±1.30^{bcAB}
3.5mg/ml	71.73±1.07^{bBCD}	51.19±1.57^{bE}	53.90±1.09^{bE}	69.58±0.98^{bBCD}	67.26±1.07^{abD}	74.40±1.30^{aAB}	74.40±1.30^{abAB}	74.26±0.91^{aABC}	78.87±1.57^{abA}	68.15±1.30^{bCD}
4mg/ml	79.17±1.07^{aA}	58.63±1.07^{aC}	60.42±1.07^{aC}	75.00±1.36^{aAB}	72.02±1.07^{aB}	79.17±0.79^{aA}	79.17±1.07^{aA}	79.17±1.07^{aA}	80.95±1.07^{aA}	77.68±2.36^{aAB}
IC_{50} mg/ml	2.40±0.06^{CDE}	3.52±0.06^{A}	3.16±0.04^{B}	2.17±0.03^{EF}	2.51±0.05^{CD}	2.62±0.02^{C}	2.36±0.09^{DE}	2.12±0.01^{F}	1.98±0.04^{F}	2.05±0.01^{F}

3.1.7 Estimation of Total Phenolic Content of Fungal Extracts

Total phenolic content was significantly different among examined extract concentrations ($p \leq 0.05$; Table 8). An apparent concentration-related increase in total phenolic content was observed for isolate P7 (2.06 ± 0.09 – 3.61 ± 0.05 mg/ml gallic acid equivalents/mg crude extract) compared to other profiled extracts. Conversely, isolate P1 exhibited significantly lower total phenolics content (2.85 ± 0.03 mg/ml gallic acid equivalents/mg crude extract), at highest concentration examined ($p \leq 0.05$; Table 8). Notably, the total phenolics correlation co-efficient (R^2) value was estimated at 0.9695 (Figure 1).

Table 8

Total Phenolic Content of Fungal Extracts

Total Phenolics (mg gallic acid equivalents/mg crude extract; mg/ml)									
Extract Conc.	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	2.03±0.03^{eBC}	1.79±0.01^{cDE}	1.58±0.03^{eE}	2.43±0.01^{eA}	2.19±0.02^{fB}	1.96±0.08^{eCD}	2.06±0.09^{eBC}	1.97±0.05^{cBCD}	2.14±0.02^{dBC}
2mg/ml	2.36±0.07^{dAB}	1.92±0.04^{cDE}	1.79±0.01^{dE}	2.50±0.03^{deA}	2.45±0.03^{eA}	2.23±0.05^{dBC}	2.43±0.04^{dAB}	2.07±0.04^{cCD}	2.32±0.03^{dAB}
2.5mg/ml	2.55±0.03^{cAB}	2.32±0.04^{bB}	1.87±0.02^{dC}	2.61±0.03^{dAB}	2.84±0.04^{dA}	2.67±0.04^{cAB}	2.84±0.07^{cA}	2.65±0.17^{bAB}	2.55±0.06^{cAB}
3mg/ml	2.60±0.01b^{cDE}	2.42±0.09^{bEF}	2.32±0.03^{cF}	3.00±0.02^{cAB}	3.04±0.02^{cA}	2.81±0.04^{bcBC}	3.08±0.03^{bcA}	3.00±0.04^{abAB}	2.70±0.04^{bcCD}
3.5mg/ml	2.74±0.03^{abDE}	2.48±0.09^{bF}	2.56±0.04^{bEF}	3.16±0.05^{bAB}	3.28±0.03^{bA}	3.05±0.02^{bBC}	3.30±0.06^{bA}	3.14±0.03^{aAB}	2.87±0.03^{bCD}
4mg/ml	2.85±0.03^{aD}	2.97±0.06^{aC}	2.92±0.05^{aCD}	3.56±0.04^{aAB}	3.49±0.05^{aAB}	3.56±0.04^{aAB}	3.61±0.05^{aA}	3.36±0.05^{aB}	3.13±0.04^{aC}

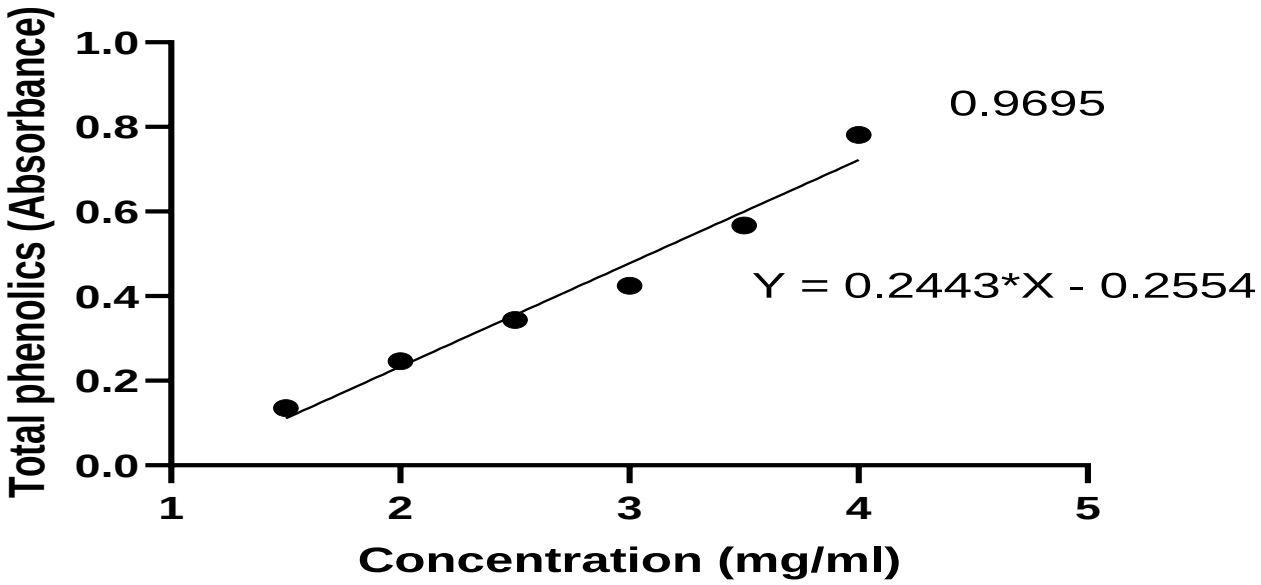

Figure 1
Estimation of Total Phenolics and Gallic Acid Equivalents (mg/ml) of Fungal Extracts.

Correlation co-efficient value R^2 = 0.9695. Each point in the calibration curve represents mean of three experiments (n=3). Reference standard (gallic acid). Total phenol content of extracts expressed in mg gallic acid equivalents/mg crude extract.

3.1.8 Estimation of Total Flavonoids Content of Fungal Extracts

As shown in Table 9, total flavonoids content of fungal extracts differed significantly in all concentrations examined ($p \leq 0.05$). The highest extract concentration yielded maximal flavonoids content compared to lesser treatment values (Table 9). Isolate P6 extracts displayed significantly higher total flavonoids content (3.32 ± 0.04 mg/ml quercetin equivalents/mg crude extract) among all extracts analysed at 4 mg/ml concentration ($p \leq 0.05$; Table 9). Contrastingly, P1 extracts demonstrated least flavonoids content (2.36 ± 0.05 mg/ml quercetin equivalents/mg crude extract) at similar concentration range ($p \leq 0.05$; Table 9). The total flavonoids correlation co-efficient value (R^2) was estimated at 0.9828 (Figure 2).

Table 9
Total Flavonoids Content of Fungal Extracts

Total Flavonoids (mg quercetin equivalents/mg crude extract; mg/ml)									
Extract Conc.	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**
1.5mg/ml	1.63 ± 0.06^{d} DE	1.39 ± 0.0 1^{cF}	1.37 ± 0.0 1^{eF}	2.16 ± 0.0 3^{eA}	1.92 ± 0.0 5^{eB}	1.82 ± 0.0 3^{eBC}	1.53 ± 0.0 3^{dDEF}	1.66 ± 0.0 4^{eCD}	1.46 ± 0.0 2^{dEF}
2mg/ml	1.87 ± 0.03^{c} C	1.44 ± 0.0 1^{cE}	1.45 ± 0.0 1^{deE}	2.39 ± 0.0 2^{dA}	2.14 ± 0.0 3^{dB}	1.91 ± 0.0 2^{eC}	1.70 ± 0.0 2^{dD}	1.92 ± 0.0 4^{dC}	1.83 ± 0.0 3^{cC}
2.5mg/ml	1.93 ± 0.03^{c} D	1.47 ± 0.0 1^{cE}	1.52 ± 0.0 2^{dE}	2.49 ± 0.0 3^{cdA}	2.29 ± 0.0 4^{dAB}	2.06 ± 0.0 4^{dBCD}	2.16 ± 0.0 8^{cBC}	2.28 ± 0.0 6^{cAB}	2.03 ± 0.0 7^{bcCD}
3mg/ml	2.02 ± 0.03^{b} cC	1.70 ± 0.0 2^{bD}	1.81 ± 0.0 3^{cD}	2.60 ± 0.0 1^{cAB}	2.49 ± 0.0 3^{cAB}	2.45 ± 0.0 2^{cB}	2.55 ± 0.0 2^{bAB}	2.63 ± 0.0 6^{bA}	2.17 ± 0.0 3^{bC}
3.5mg/ml	2.16 ± 0.05^{b} C	1.86 ± 0.0 5^{bD}	2.09 ± 0.0 5^{bC}	2.80 ± 0.0 2^{bAB}	2.82 ± 0.0 3^{bAB}	2.83 ± 0.0 3^{bA}	2.81 ± 0.0 3^{aAB}	2.73 ± 0.0 2^{bAB}	2.64 ± 0.0 2^{aB}
4mg/ml	2.36 ± 0.05^{a} E	2.53 ± 0.1 0^{aDE}	2.51 ± 0.0 4^{aDE}	3.31 ± 0.0 5^{aA}	3.21 ± 0.0 6^{aAB}	3.32 ± 0.0 4^{aA}	2.95 ± 0.0 5^{aBC}	3.10 ± 0.0 6^{aAB}	2.74 ± 0.0 7^{aCD}

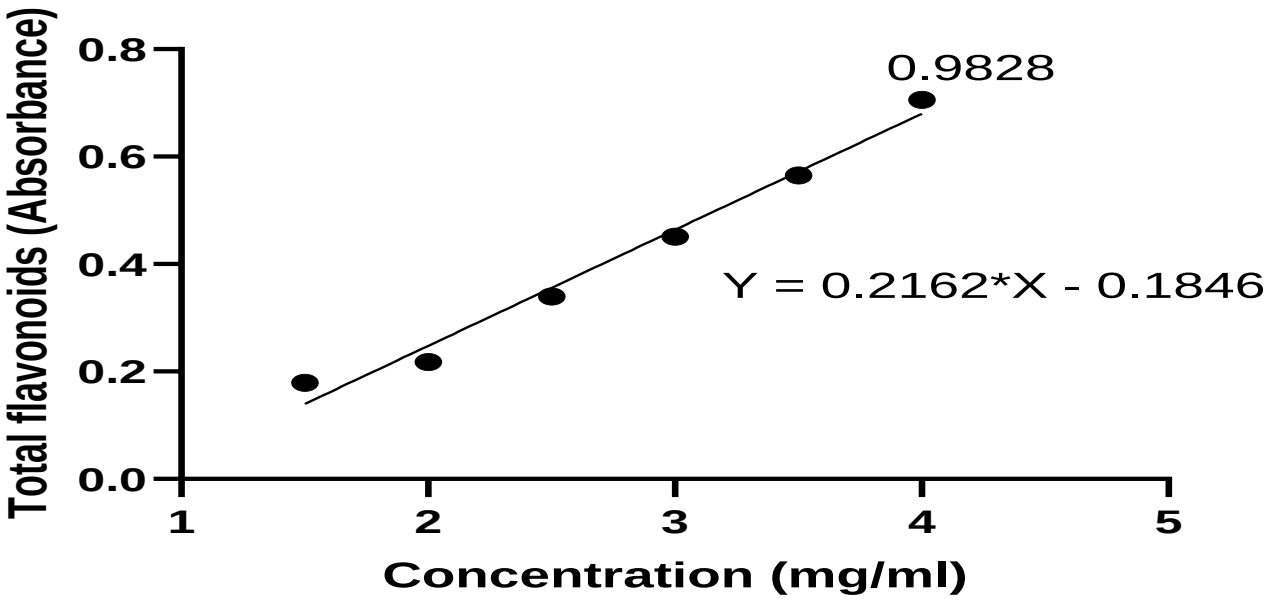

Figure 2
Estimation of Total Flavonoids and Quercetin Equivalents (mg/ml) of Fungal Extracts

Correlation co-efficient value R^2 = 0.9828. Each point in the calibration curve represents mean of three experiments (n=3). Reference standard (quercetin). Total flavonoid content of extracts expressed in mg quercetin equivalents/mg crude extract.

IV. DISCUSSION

Study isolates recovered from Lake Magadi, Kenya are comparable to fungal genera previously isolated from saline-alkaline habitats that appear to share ecological preferences to extreme natural conditions. Numerous *Aspergillus, Cladosporium* and *Penicillium* strains exerting dominance and higher colonisation have been isolated from alkaliphilic and hypersaline settings of varying geographical localities including waters of *Chott* Melghir (Algeria), Wadi Natrun (Egypt), Lake Bogoria (Kenya) and Solar salterns (Puerto Rico) (Dendouga and Belhamra, 2022; Moubasher *et al.*, 2018; Salano *et al.*, 2017; Wingfield *et al.* 2023). Salinity influences both chemical and biological processes in aquatic and terrestrial habitats and is previously shown to impact positively on growth and survivability of diverse microbial biota including extremophilic fungal species in hypersaline ecosystems (Venkatachalam *et al.*, 2019). Fungal sporulation, enzymes and extrolites expression is intensified in NaCL and glucose enriched catchments (Tepsic *et al.*, 1997). Significantly higher rates of enzymes and metabolites production heightens tolerability against osmotic stress (Raddadi *et al.*, 2015).

More importantly, the levels of salt tolerance modulating fungal medial growth and differentiation characteristics was factored in the current study at 3.5% NaCL, which is comparable to previous studies that reported optimal media fungal growth and isolation at salt tolerance levels of between 2 – 10% (Jaouani *et al.*, 2014; Ndwigah *et al.*, 2015). Likewise, assayed fungal isolates were predominantly alkaliphiles and seldom possess acidophilic properties, that may be attributable to adaptive resilience exhibited by alkali-tolerant fungal community species against ecological stressors within saline-alkaline biodiversities (Orwa *et al.*, 2020).

The use of SDA, PDA and MEA fungal propagation media at RT and 41^0c conforms to former studies using similar media types for preliminary fungal growth and isolation from saline-alkaline habitation (Salano *et al.*, 2018; Moubasher *et al.*, 2018). The assorted media types are demonstrated to accelerate heightened isolation and cultivation of yeasts and molds from varied ecological sources while permitting expression of prospective fungal phenotypes (Acharya and Hare, 2022). A substantial proportion of halotolerant and alkalophilic microbial species have revealed optimal growth and isolation at temperature ranges between 20 – 45^0c (Dendouga and Belhamra, 2022). Sample types composed of wet sediments and soils showcase optimal retrieval of diverse fungal species owing to significantly high nutrient levels, ionic and trace elements, dissolved oxygen and tolerable pH levels influencing abundance of diverse microbial biota (Fazi *et al.*, 2018).

Bioprospecting anti-oxidants from natural ecosystems stems from proven adverse effects and toxicity linked to synthetic antioxidants (Kobets *et al.,* 2022). Fungi from haloalkaliphilic ecological settings signify a rich but less exploited reservoir of chemo-constituents with potential for anti-oxidant bioactivity (Vladkova *et al.,* 2022). Cinnamic acid from *C. cladosporioides* extracts donates electrons that react with free radicals to form stable products (Ruwizhi & Aderibigbe, 2020). Cladosporin D sourced from *Cladosporium* broth extracts optimally scavenges DPPH radicals with notable IC_{50} value of 16.4 µM (Amin *et al.,* 2020). Our study findings tally with former reports showcasing marked DPPH and NO radical scavenging action alongside ferric (Fe^{3+}) ion reducing power in *A. fumigatus* broth extracts (Arora and Chandra, 2011). Yet still, *A. fumigatus* extracts are previously described to express emodin that actively scavenges superoxide radicals (Izhaki, 2002; Rossi *et al.,* 2020).

Moreover, emodin may partially be attributed with *in vitro* lipid peroxidation inhibitory activity of isolate P5 extracts in this study. Arguably, the premise aligns with prior reports revealing protective role of emodin from damaging effects of lipid peroxidation (Manimaran & Manoharan, 2018). Antilipid peroxidation activity parallels reduction in MDA levels that reflects upon active preservation of cell membrane constituents (Cordiano *et al.,* 2023). Scavenging of OH radicals by P2 and P4 extracts stems from array of extrolites expressed by wide-ranging *Fusarium* strains. Fusarubin and anhydrofusarubin obtained from *Fusarium* sp. extracts exhibit substantial free radical scavenging effectiveness with IC_{50} values of 34.8 µg/ml and 12.4 µg/ml against ascorbic acid; 1.5 µg/ml (Khan *et al.,* 2018). Fusarubin's radical scavenging action entails NADH ubiquinone reductase inhibition that disrupts electron transport chain and proton gradient across the mitochondrial membrane (Albracht *et al.,* 2011).

Furthermore, isolates P5, P6 and P7 extracts' H_20_2 radical scavenging capacity conforms to prior studies revealing potential of genera *Aspergillus* and *Cladosporium* to scavenge and neutralise free radical molecules (Alam and Kataria, 2021). The scavenging action of *Aspergillus* sp. for the above-listed reactive by-product emanates from presence of terreic acid and terremutin (Dewi *et al.,* 2012). The quinone epoxide (terreic acid) antioxidation activity is credited to quinine group acting as a single electron acceptor besides free radical chain-breaker through reaction with alkyl radical (Dewi *et al.,* 2012). *Cladosporium* sp. extracts express silver nanoparticles (AgNPs) alongside coumarin compounds that possess potent H_20_2 scavenging and Fe^{3+} reductive action (Prahadeesh *et al.,* 2018; Hulikere and Joshi, 2019).

Total phenolic content correspondent to gallic acid equivalents revealed a concentration-based relationship with superior phenolic levels observed in isolates P4, P6 and P7 extracts. Findings align with preceding experimental bioassays positively correlating the scale of fungal antioxidation activity with total phenolic content among profiled compounds (Arora and Chandra, 2011; Couttolenc *et al.,* 2022). Phenolic derivatives such as cinnamic acid and p-toluic acid have formerly been detected in fungal fermentation extracts at appreciable levels (Almatar and Makky, 2016; Sallam *et al.,* 2023), which may justify the substantial total phenolic content and higher antioxidant activity observed in profiled fungal isolates. Structurally, the number and positioning of hydroxyl groups on the phenol ring considerably impacts on redox properties and cumulative antioxidant effectiveness of phenolic acids (Khoddami *et al.,* 2013). Nonetheless, the exclusive feature of chelating transition metals including iron by polyphenolic compounds (Pan *et al.,* 2022), may boost understanding of the Fe^{3+} reducing antioxidant ability observed with our assayed study extracts.

Total flavonoids content equated with quercetin equivalents showed a concentration related relationship, with higher flavonoids content exhibited by isolates P4, P5 and P6 extracts. Despite this study seldom classifying distinct flavonoids, there exists verifiable concept that phenolic acid effects can similarly be extended to their flavonoid counterparts (Zhang *et al.,* 2022). Structurally, bioflavonoids are categorised among polyphenolic compounds alongside phenolic acids, tannins, lignans and stilbenes (Ayad and Akkal, 2019). The inference is supported by a shared chemical structure encompassing an aromatic ring with added hydroxyl substituents (Aludatt *et al.,* 2017). Summatively, these convergent considerations cement the link between demonstrated antioxidation activity and total flavonoid content occasioned with our study extracts.

Nevertheless, it is vital to acknowledge that this study had some limitations. The qualitative and quantitative metabolomic constituents expressed by haloalkaliphilic study isolates were not evaluated. Additionally, seasonal variability patterns that may influence total fungal community were not critically evaluated within our study. Collectively, the variables may provide wider scope in elucidating mechanisms of antioxidation activity as well as diversity of fungi biota within our study setting.

V. CONCLUSIONS & RECOMMENDATIONS

5.1 Conclusion

Study findings provide scientific validation for presence of antioxidation capacity in fungal extracts from Lake Magadi, Kenya. These may further be exploited for disease fighting capabilities that informs their potential use medicinally for management of oxidative stress related maladies.

5.2 Recommendations

Our study recommends further purification of fungal extracts through bioassay targeted fractionation accompanied by chromatographic and mass spectrometric screening of bioactive metabolomic constituents. Need exists for adoption of animal driven experimentation models using *in vivo* and *ex vivo* modalities to elucidate underlying mechanisms of antioxidation activity.

Acknowledgements

We are grateful to Kenya Wildlife Service (Ref. KWS/BRP/5001), County Government of Kajiado (Ref. KJD/CC/ADM/45 Vol.I1 (172) and Tata Chemicals Magadi limited for authorizing access to Lake Magadi for sampling. Research permit was sought from National Commission for Science, Technology and Innovation (License No. NACOSTI/P/19/621). Financial support from National Research Fund (NRF) Kenya and South Eastern Kenya University (SEKU) internal grants (Ref. SEKU/ARI/DRIC/019 Vol. I) were greatly appreciated. Notably, the funders did not influence the drafting, review and validation of the manuscript.

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