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Antioxidant, Antifungal and Cytotoxic Impact of *Acacia nilotica* Bark Aqueous Extract

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Abstract: The medicative plants were copiously applied in the healing of health complications. Traditionally implemented, medicative-worthy *Acacia nilotica* (Fabaceae family), is applied globe-wide in customary healing practices. Here, we have tried to unravel the antioxidant, fungicidal, antibacterial and cytotoxic trait of *A. nilotica* bark aqueous extract. Phyto-content testing revealed presence of flavonoids, tannins, alkaloids, phenols, carbohydrates, proteins, and amino acids. The antioxidant efficacies were 39.12 µg/ml (for DPPH assay) and 42.64 µg/ml (for NO assay) as IC₅₀ ranges, respectively. The bark aqueous extract manifested fungicidal impact on all the screened fungal strains with the highest inhibitory zone against *A. fumigatus* (19.5 ± 0.45 mm). The MTT assay of bark aqueous extract registered a dose-related cytotoxic impression with 39.05 µg/ml (IC₅₀ outcome). Hence, it emphasizes strongly that *A. nilotica* bark aqueous extract is a prospective therapeutic source and it is indispensable to further scrutinize its therapeutic and curative properties.

Keywords: *Acacia nilotica*, Phyto-content, Antioxidant, Fungicidal, Cytotoxic

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Introduction

Plants embracing recuperative traits have been the chief consideration resource due to their medicament-bearing chemically diverged and bioactive moieties (Mathur and Hoskins, 2017). Historically, plant-acquired medicaments have been recommended and applied extensively in

therapeutics and obviating human maladies (Licciardi and Underwood, 2011). The statistical outcome of WHO (World Health Organization) evinced that an extensive percentage (80%) of humans residing in the economically emerged as well as developing nations are entirely rooted in

customary medicaments to address their basic health-care necessities (Chaachouay *et al.*, 2022). Scrutiny and standardization of the bioactive phyto-content in medicative plants are pivotal for measuring their pharmacological goodness. This will aid in the resurgence of framing innovative therapeutic medications (Eswaraiah *et al.*, 2020; Sharma *et al.*, 2021).

The diverged classic system of Indian medicine predominately avails medicinally indispensable plants as native drugs and amid all, a holistic mode is bestowed by the Ayurvedic system (Mukherjee *et al.*, 2012). Nowadays, pharmaceutical trades apply bioactive phyto-content for mitigating metabolic complaints owned to their lower aftereffect, low-priced and procurable mode as an efficient regimen, contrary to the allopathic medicaments which had laid intricate formulation protocols, inefficacy and aftermath complaints (David *et al.*, 2015; Birjees *et al.*, 2022).

The creation of free radicals ensues in the dwelling aerobic system as a consequence of cellular respiratory responses in a milder range. Disproportionality between the mechanism of oxidants and the endogenously pertaining antioxidant system as an outcome of oxidative stress proliferates the unmanageable creation of free radicals, mentioned as reactive oxygen species (ROS). This leads to complicated long-term medical disorders such as cardiovascular issues, arthritis and diabetes mellitus (Hmidani *et al.*, 2019). Besides, the external boosters of free radical genesis include environmental pollutants, chemical pesticides and tobacco smoke. These factors also tune up the phagocytic mechanism drastically in the cellular interior causing plenteous ROS genesis (Karavitis and Kovacs, 2011; John and Souza, 2017). Phytoantioxidants with precise redox efficacy manifest astounding antiradical attributes and are implemented for ameliorating health disturbances (Calegari *et al.*, 2020).

Even though the pharmaceutical sectors are working continuously to offer drugs holding novel therapeutic traits, incurable infections are still

enduring as the deadliest diseases, apart from other non-infectious diseases (Talebi Bezmin Abadi *et al.*, 2019). The misutilization and overexploitation of anti-infective drugs (antibiotics) have prompted stubbornness in the infective classes of microbes towards the antibiotics (Iyigundogdu *et al.*, 2017). Additionally, the practice of antibiotic in the food offering animals and agriculture has further proven antibiotic-stubbornness in microbes through gene-resistant mechanisms (Levy and Bonnie, 2004). With the present reality of antibiotic-stubbornness strains, the exigency of averting this challenging crisis globally has become an imperative consideration (Prestinaci *et al.*, 2015). Nowadays, the opportunistic occurrence of infections by fungal strains has prevalently escalated, turning into the key root of infections with the non-success of antifungal medicaments (Abirami *et al.*, 2021). Immunocompromised populations are usually the targets of recurrently acquired newer fungal infections, thus creating a critical incidence of morbidity along with mortality around the globe. Fungal-emerged dermal diseases (dermatophytosis) and other forms of infections are the pervading contagious infective disease and their progressiveness has been dramatically visualized in the course of the last ten (Zanna *et al.*, 2021).

Cancer is a chief leading reason for global death, which has been constantly upsurging. This disease entails the continuous proliferation of abnormal cells owned by the ungovernable cellular cycle progression, consequently eliciting the origination of tumorous cellular development (Ohiagu *et al.*, 2021). As per the epidemiologically published statistics, globally, breast cancer remains the utmost-ranked root of mortality in women, in contrary to other cancer types (Azamjah *et al.*, 2019) and approximately one woman out of ten was spotted with breast cancer as surveyed by WHO (Nunes *et al.*, 2018). The cytotoxic impression of chemotherapeutic regimens is facing the resistibility of MDR (multidrug-resistance) cells. One of the practicable perspectives to tackle the above-mentioned issue

is to develop puissant antineoplastic drugs (Alasmary *et al.*, 2018) as the chemically procured anticancer medicaments are stuffed with the aftermath impacts of distorting healthier cells and lower biological availableness. So, alternatively procured bioactive chemical ingredients from the plant's various sections are in trials to bring better anticancer regimens (Iqbal *et al.*, 2017).

Acacia nilotica is a multi-utility tree belonging to the genera of Acacia and Fabaceae family. In native words, it is mentioned as Babul or Babool. It has not only an ornamental impression but also pertains to restorative worthiness. It is noticeably scattered throughout the entire globe, specifically in the tropical parts as well as sub-tropical parts. Also, holds nativity to Egypt, America, India and America (Saeedi *et al.*, 2020). In the Indian Ayurvedic healing system, the leaves, pods and barks were served against gastrointestinal troubles (diarrhea), menstrual issues, fever, metabolic diseases (cancer and diabetes mellitus), gallbladder issues, smallpox and respiratory-related diseases (cold and tuberculosis). Even the paste made from the bark is applied to strengthen oral health (teeth and gums) (Majeed *et al.*, 2021). The decoction acquired from the bark is applied in mitigating sore throat complaints. Also, the bark is extensively employed as a mouth cleaner in commercially available toothpaste (Fadhil *et al.*, 2021). Previously, scrutinized pharmacological outcomes had authenticated anti-inflammatory, antimicrobial, antidiuretic and antipyretic traits in the various organs of this tree (Ali, 2012; Roozbeh *et al.*, 2017).

Materials and Methods

Source, identification and processing of bark material of A. nilotica:

Mature as well as healthy bark pieces from the *A. nilotica* tree were collected from Kalapadi Village, Gudiyattam, Vellore district in August 2022. The scrapped bark from the tree was placed in a sterile plastic bag and taken for analysis purposes to the research laboratory. A voucher deposition of *A. nilotica* with the accession number PARC/2021/4512 including the chosen plant's

recognition characteristics was executed by the Plant Anatomy Research Centre (PARC) of West Tambaram, Chennai (Tamil Nadu), India.

Bark material drying and processing:

The methodology along with the drying conditions acquired may render a credible impression on preserving chemical entities within the medicinally worthy plant samples. The surface grime from the bark of *A. nilotica* was taken out with surplus running tap water and rinsed thrice in distilled water. The damaged portions of the bark, inclusive of the part infected by the fungus, were also manually eradicated. The healthy bark was shade-dried for a couple of weeks over clean paper by evenly spreading to remove the moisture content and attain a constant mass. Subsequently, the dried bark was incised into tiny pieces, powdered in a home blender, and sieved to acquire a finer powdery bark content. Then the desired powdery bark content was placed in an airtight bottle container at ambient temperature and used in all experimentation work.

Preparation of aqueous bark extract of A. nilotica:

500 g of *A. nilotica* bark powder were efficaciously loaded in the thimble of the extractor of Soxhlet equipment and extraction was executed using polar solvents (water). The darkish-coloured marc procured was concentrated through a vacuum within a rotary evaporator, dried and subsequently, maintained at 4°C (refrigerated) in a sterile vial for various further tests.

Phyto-content of A. nilotica bark aqueous extract:

To spot the bioactive phyto-content in the *A. nilotica* bark acquired aqueous extracts (crude extract), various chemical tests as per the availed standard protocols were employed (Iqbal *et al.*, 2015; Choudhary *et al.*, 2021).

Antioxidant assessment:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assessment:

The chemically stable, DPPH (2,2-diphenyl-1-picrylhydrazyl) protocol was taken as already

executed (Clarke *et al.*, 2013) with slight variations. Varying dosages (20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml and 60 µg/ml) of one milliliter of *A. nilotica* bark aqueous extracts were mingled with 0.004 % methanolic DPPH solution (4 ml). In a dark area for 30 min, the reaction solution was placed at ambient temperature. The absorbance of the yellowish chromophore was measured spectrophotometrically at 517 nm. Methanol was utilized as a blank whereas, ascorbic acid was applied as a positive experimental control. The inhibition of DPPH radicals by *A. nilotica* bark aqueous extract was computed and presented as the percentage by implementing the following formula:

$$\text{DPPH radical \% inhibition} = [(C_{(OD)} - S_{(OD)})/C_{(OD)}] \times 100$$

Here, $C_{(OD)}$ = Absorbance (methanolic solution of DPPH) as control, $S_{(OD)}$ = Test sample (Bark aqueous extract/Ascorbic acid) absorbance including DPPH. The IC_{50} ranges were also identified.

Nitric oxide (NO) scavenging assessment:

Griess test was applied (Sylvie *et al.*, 2014) to assess the nitric oxide radicals created by sodium nitroprusside aqueous solution. For this assay, *A. nilotica* bark aqueous extract (1 ml) with diverse dosage ranges (20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml and 60 µg/ml) was stirred with 2 ml (10 mmol/l) of sodium nitroprusside prepared in 50 mmol/l phosphate buffer (pH 7.4) and retained at 37°C for 150 min. To the reaction solution (0.5 ml), 1 ml sulfanilic acid was added and allowed for diazotization, subsequently added 1 ml of 0.1 % naphthylethylenediamine dihydrochloride. The pinkish chromophore acquired after half an hour of incubation was noticed at 540 nm by placing the solutions in a spectrophotometer. Ascorbic acid was applied as a positive experimental control. The percentage (%) reduction of NO radicals by the bark acquired aqueous extract was computed and presented by implementing the following formula:

$$\text{NO radical \% inhibition} = [(C_{(OD)} - S_{(OD)})/C_{(OD)}] \times 100$$

Here, $C_{(OD)}$ = Control absorbance, $S_{(OD)}$ = Test sample (Bark aqueous extract/Ascorbic acid) absorbance. The IC_{50} ranges were also identified.

Antifungal assay of *A. nilotica* bark aqueous extract:

In vitro execution of agar-well diffusion protocol to assess the fungicidal efficacy of *A. nilotica* bark aqueous extract was done against *Aspergillus fumigatus* (*A. fumigatus*; MTCC-9657), *Candida tropicalis* (*C. tropicalis*; MTCC-4690), *Aspergillus flavus* (*A. flavus*; MTCC-873) and *Candida albicans* (*C. albicans*; MTCC-4748). The infective fungal strains were gently swabbed onto the PDA (potato dextrose agar) solidified medium in a petri-plate. Applying a sterile steel 6 mm width borer, the wells were created in the solidified PDA medium. Varying dosages (10 µg/ml to 40 µg/ml) of aqueous bark extract were poured into each well of individual plates with the aid of a micropipette. After incubation (2 days) at 37 °C, the width of the appeared inhibition zone was carefully measured as millimeter (mm) and the outcomes were registered. Amphotericin B (10 µg/ml) was chosen as a positive experimental control (Ferin Fathima *et al.*, 2020).

Cytotoxic screening of *A. nilotica* bark aqueous extract:

MCF-7 cells (breast cancer cell line) acquired from the National Centre for Cell Science (NCCS), Pune, India, were maintained in high humidity (95%), 5% of carbon dioxide (incubation condition) and provided with DMEM (Dulbecco's modified Eagle's medium) in addition to 10% of fetal bovine serum and 1% w/v of antibiotic (penicillin).

Cell viability determination by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] assay:

In vitro assessment of the cytotoxic efficacy of *A. nilotica* bark aqueous extract on the cellular viability of MCF-7 cells was performed (Menon and Shanmugam, 2020). Once the MCF-7 cells (1 x 10⁵ cell density/well) loaded in the individual well of 96 well microplates attained confluency (after 24 h), they were exposed to *A. nilotica* bark

Table 1: The dynamic secondary metabolic and other phyto-content spotted in the *A. nilotica* bark aqueous extract

Phyto-content	Executed test	Acquired observation	Inference
Flavonoids	Lead acetate test	Yellowish coloured precipitation	Moderately evidenced
Tannins	Ferric chloride test	Deep darkish-blue colouration	Copiously evidenced
Alkaloids	Wagner's test	Red to brownish precipitate	Moderately evidenced
Phenols	Ellagic acid test	Muddy brownish colouration	Moderately evidenced
Carbohydrates	Molisch test	Violet coloured ring formed between the two layers of liquid	Mildly evidenced
Proteins	Biuret test	Violet chromophore appeared	Mildly evidenced
Amino acids	Millon's test	Darkish red colouration identified	Mildly evidenced

aqueous extract (10 µg/ml to 90 µg/ml). Again, incubated for 24 h and by applying PBS (phosphate buffered saline), the cells were washed. Exactly, 0.5% of MTT (100 µg/ml) was loaded into individual culture wells and retained for 4 h. Then the solution of MTT was drained out from the wells. The procured formazan crystals with purplish colouration were diluted in 100 µg/ml of DMSO (dimethyl sulfoxide) solution and registered the absorbance intensity at 570 nm in an ELISA microplate reader. As negative reference (control), *A. nilotica* bark aqueous extract unexposed MCF-7 cells were taken. The cellular viability (%) was provided as--

Percentage (%) in the cellular viability = [Absorbance noted for *A. nilotica* bark aqueous extract exposed MCF-7 cells/Absorbance noted for the control cells] x 100

Statistical analysis:

Each experimentation was executed in three trials separately and the outcomes were conveyed in descriptive statistics (mean ± standard deviation) applying GraphPad Prism (version 8.4.2). In the numerical data, the p (probability) value of < 0.05 was taken as statistically significant executing the student's t-test.

Results

Phyto-content identification:

The phyto-content recognized through the chromogenic testing of *A. nilotica* bark aqueous

extract unfolded the existing medicinally appreciable secondary metabolites (flavonoids, tannins, alkaloids, phenols, carbohydrates, amino acids as well as proteins) (Table 1).

Antioxidant assessment:

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assessment:

Exhibition of exemplary antioxidant activity occurred with the increased dosage of the *A. nilotica* bark aqueous extract with descending absorbance at 517 nm. At a higher applied concentration (60 µg/ml), the inhibition of DPPH radical by *A. nilotica* bark aqueous extract was the highest and at the milder concentration (20 µg/ml), the inhibition (%) of DPPH by the bark extract was lesser. The IC₅₀ value was 39.12 µg/ml for *A. nilotica* bark aqueous extract and 31.05 µg/ml for the positive experimental control (Ascorbic acid). The outcomes of the DPPH radical inhibitory percentage by *A. nilotica* bark aqueous extract are displayed in Figure 1.

Nitric oxide (NO) scavenging assessment:

A. nilotica bark aqueous extract confirmed dose-responsive activity. At 20 µg/ml to 60 µg/ml, the nitric oxide scavenging property of *A. nilotica* bark aqueous extract as percentage inhibition was gradually escalated. An IC₅₀ value of 42.64 µg/ml was achieved for *A. nilotica* bark aqueous extract.

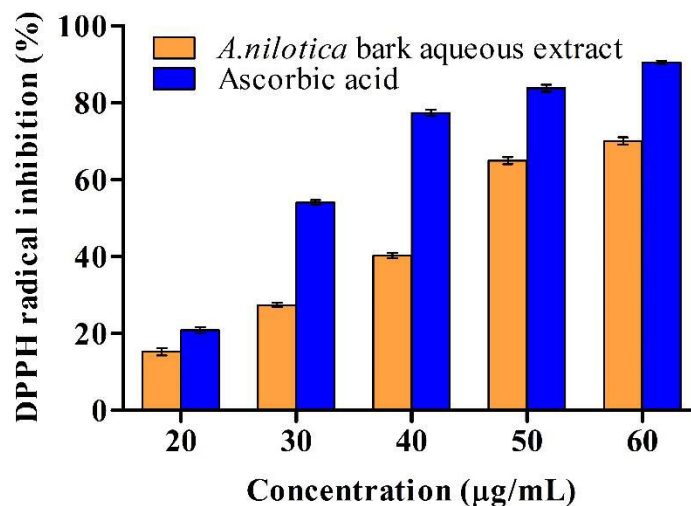


Fig. 1: DPPH radical % inhibition by *A. nilotica* bark aqueous extract.

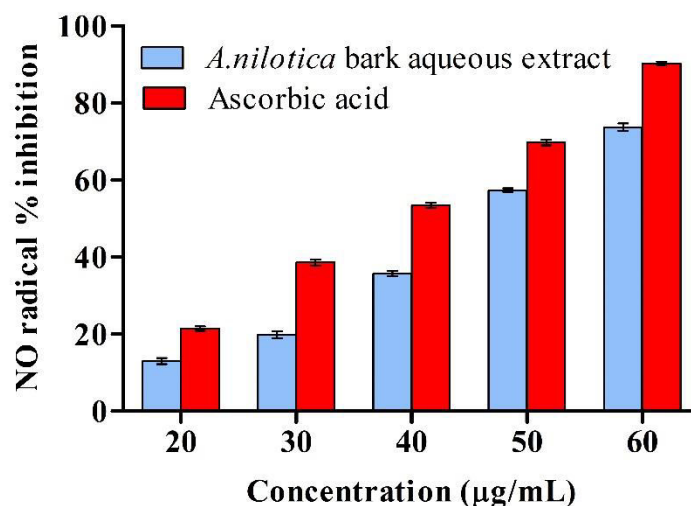


Fig. 2: NO radical % inhibition by *A. nilotica* bark acquired aqueous extract.

Ascorbic acid presented an IC_{50} value of 35.50 µg/ml. The bark extract elucidated commendable radical reductive potential comparable to ascorbic acid ultimately, making *A. nilotica* a medicinally worthwhile tree (Fig. 2).

Antifungal assay of A. nilotica bark aqueous extract by agar-well diffusion:

The executed antifungal screening employing agar-well diffusion protocol gave astounding fungicidal activity through the generation of ZOI (zone of inhibition) around each well of the

scrutinized fungal strains. The antifungal activity was entirely dosage-based form. With the upsurge in the dosage ranges of *A. nilotica* bark aqueous extract, the fungicidal activity was also enhanced. At the screened maximum dosage (40 µg/ml), the ZOI registered as 19.5 ± 0.45 mm (*A. fumigatus*), 19.3 ± 0.41 mm (*C. tropicalis*), 18.9 ± 0.20 mm (*C. albican*), and 17.0 ± 0.00 mm (*A. flavus*) (Fig. 3).

MTT assay:

The cytotoxic impression of *A. nilotica* bark aqueous extract was scrutinized by MTT protocol

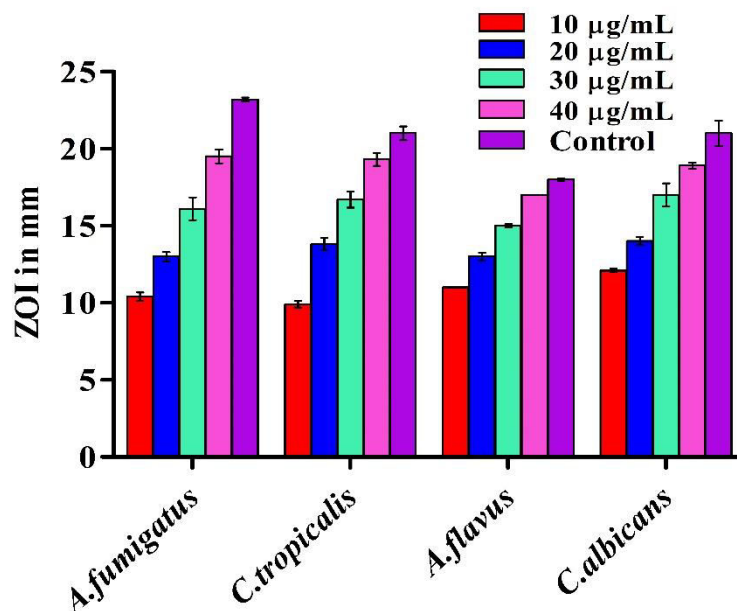


Fig. 3: Antifungal activity testing outcomes of *A. nilotica* bark aqueous extract against the randomly taken pathogenic fungal strains.

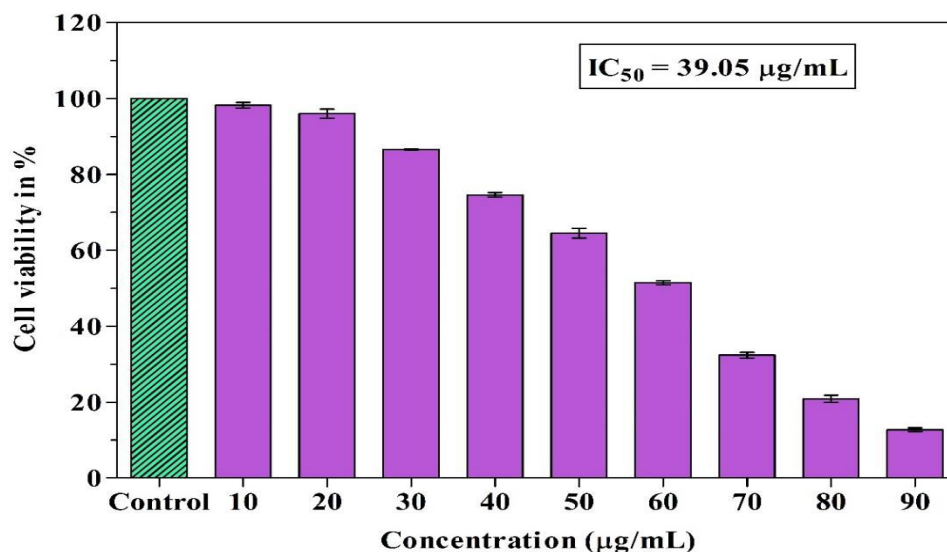


Fig. 4: MTT assay of *A. nilotica* bark aqueous extract against screened MCF-7 cell (breast cancer cells) line.

on the breast cancer cell line (MCF-7 cell line). Nine varying dosages of *A. nilotica* bark aqueous extracts were applied and showed dose-based growth-inhibitory impact on the MCF-7 cells after

24 h of exposure. The inhibition was milder (98.21%) at 10 µg/ml and greater (12.68%) at 90 µg/ml. The IC_{50} value noted was 39.05 µg/ml (Fig. 4).

Discussion

The phyto-content displays magnificent antioxidant activity and also executes an appreciable reflection of its pharmacological functions. The plant availed secondary dynamic metabolites owns the traits to diminish the incidence and progression of metabolic complaints such as neurological, cardiovascular and cancer diseases (Saravanan *et al.*, 2018). The chromogenic test implemented spotted phytochemicals that included flavonoids, tannins, alkaloids, phenols, carbohydrates, proteins, as well as amino acids. The outcomes were consistent with the formerly identified and published experimental reviews (Sawant *et al.*, 2014).

Antioxidants are a vital chemically natural substance with the impact of suppressing the oxidative type of reactions and declining the oxidative form of cellular damage by diminishing ROS creation. Antioxidants sustain good health by preventing DNA and protein distortion, which are the major culprits for metabolic complaint (Karuna *et al.*, 2018). The richness of a diet with antioxidant boosts the defensive antioxidant mechanism. The noxious impact of synthetic forms of antioxidants incorporated into commercial foods such as the risk of tumors has shifted people to intake plant-originated antioxidant-stuffed foods. Amid plants, medicative plants hold a richness in antioxidants as polyphenolic ingredients (Kumar *et al.*, 2013). Studies have proclaimed beneficiary impressions of plant polyphenolic compounds on long-lasting metabolic issues when consumed for a longer term and presently applied as food additives or nutraceuticals (Zhang *et al.*, 2022).

The potentiality of *A. nilotica* bark acquired aqueous extract to quench the free radicals was checked by executing the DPPH (a stable form of free radical) assay. This assay is the simplest and regularly adopted protocol for scrutinizing the radical quenching ability of the bioactive moieties of medicinally-worthy plants. The purplish-coloured methanolic DPPH solution was bleached to a yellowish chromophore due to the

electron/hydrogen donation by the compounds with antioxidant traits (Govindan and Muthukrishnan, 2013). The antiradical ability was entirely owned to the total phenolic content, elucidating parallelism between the radical quenching trait and phytophenols (Singh and Arora, 2009; Agrawal *et al.*, 2010). Profiling the antioxidative trait of plant extract had brilliantly delineated upsurged antioxidant activity creating a nullifying impact on the free radicals. This was thoroughly owned to the condensed form of tannin. Further, authenticated the favourable and sustainable link between the tannins and quenching of DPPH free radicals (AlMousa *et al.*, 2022).

The nitric oxide (NO) free radicals fabricated within the mammalian tissues have beneficiary charges such as relaxing the smooth muscles, messenger in neuronal communication and hindering platelet aggregation. Also, microbicidal and cytotoxicity impressions was noticed. Overstocking of nitric oxides has a baneful impression on cellular functioning and incites chronic complaints (Tuteja *et al.*, 2004; Jimoh *et al.*, 2019). In reaction with another free radical (superoxide) within the epithelial cells, they may turn up oxidative-based DNA structural distortion due to the genesis of peroxynitrite (ONOO⁻) (Jimoh *et al.*, 2019). In the executed reaction, sodium nitroprusside applied originated nitric oxide (unstable form) creates nitrate (stable form) by interacting with molecular oxygen. Plant antioxidants competed with the molecular oxygen and hindered the genesis of nitrite which was efficaciously registered at 540 nm spectrophotometrically through the Griess reaction (Ahmad Wani and Tirumale, 2018).

In the executed antifungal assessment, *A. nilotica* bark aqueous extract exhibited fungicidal trait against *A. fumigatus*, *C. tropicalis*, *C. albicans*, *A. niger*, *E. floccosum*, *R. stolonifer* and *A. flavus*. The acquired results agreed with the already declared outcomes where the water extract of *A. nilotica* exposed fungicidal features against *C. albicans* and *E. floccosum* (Kagne and

Rajbhij, 2019). The stem-bark petroleum-ether and ethyl acetate extract acquired from *A. nilotica* were tremendously active against *A. niger* and *C. albicans*. The methanolic extract was found to be inefficacious in displaying inhibitory impressions on the screened fungal strains (Ali *et al.*, 2018). The fungicidal efficacies of phyto-extracts are principally attributed to the capacity of the solvent to extract the components and the bioactive ingredient concentration in the crude phyto-extracts (Gacem *et al.*, 2019). The fungicidal activity of the plant-acquired bioactive metabolites is also own to the inhibitory impact on fungal spore germination and hindering the growth of fungal mycelium. Additionally, distorted hyphae morphology with degraded cell wall, inclusive of swelling, collapsing and shrinking occurred (Balkan *et al.*, 2017). Furthermore, the sensitivity, as well as intolerability of fungi to the phyto-contents, tunes up the inhibition in the cell wall genesis, DNA duplication, cell proliferation, protein synthesis and dysfunction of mitochondria (Lagrouh *et al.*, 2017).

Ergosterol is the principal sterol ingredient in the cell membrane of fungi pivotal for its integrity (Andargie and Li, 2019). The antifungal regimen (amphotericin B and nystatin) usually bound to the ergosterol and sprouts the cellular membrane disruption (Carvalho *et al.*, 2018). Similarly, phenolic ingredients (tannin and gallic acid) on binding to fungal cell create pores or hinder the creation of ergosterol. This is also one of the prime operative mechanisms depicted for fungal lysing. Copious origination of ROS by elicited functioning of mitochondrial respiratory dehydrogenase enzyme by the tannins leads to a noxious impact on the cellular division. The cellular free radical inactivating system becomes incapable of coping with ROS (Lopes *et al.*, 2013). ROS created peroxidation reaction of fungal cellular membrane component (phospholipid) also triggers the reduced membrane fluidity and upsurged permeability bringing fungal killing trait. Flavonoids identified (+)-catechin-5-gallate, (+)-catechin 4',5-digallate, kaempferol and (+)-catechin-3',5-digallate in the bark aceton

extract of *A. nilotica* substantiated their antifungal property (Malan, 1991; Aboody and Mickymaray, 2020). Alkaloids also dictated observable fungicidal outcomes (Singh *et al.*, 2020).

The antiproliferative trait of *A. nilotica* bark aqueous extract was checked through the MTT protocol. This protocol specifically assesses the cytotoxic impact of phyto-contents on metabolically viable cells and their proliferation. Here, the MTT (yellowish tetrazolium salt) was reduced to non-polar formazan crystals (purplish coloured crystals) by the metabolically active mitochondrial dehydrogenase enzyme (Oh and Hong, 2022). The formazan produced through the reductive mechanism has a straight relation to the cellular viability, which is spectrophotometrically registered at 570 nm. Only the viable cell retains mitochondrial intactness. So, MTT assesses only the cells that remain alive after induced cellular killing (Rai *et al.*, 2018). The explored *A. nilotica* bark aqueous extract cytotoxicity on the MCF-7 cell line was a dose-responsive type. Diverse plant ingredients were recognized for their cytotoxic traits on the cancerous cells by tuning either apoptotic or necrotic routes (Al-Oqail, 2021). The synergistic impact on cancerous cells is imposed by the diverged phyto-contents as a phytotherapy for cancer treatment. The phyto-extract biocomponents richness targets various cancer-inducing routes in contrary to the therapy by a single component (Tor *et al.*, 2015). Phyto-extract polyphenols (steroids, glycosides, tannins and terpenoids) have attained popularity owned to arrest of the cancerous cellular cycle, signalling route alteration and induction of apoptotic stages in breast cancer, colon cancer, prostate cancer, cervical cancer, lung cancer, retinoblastoma and colon cancer when screened both *in vivo* and *in vitro*. Also, polyphenolic components interfere with the cell cycle regulatory enzymes (Bhosale *et al.*, 2020).

Conclusion

All the executed protocols dictated the antioxidant, antifungal and cytotoxic impact of *A. nilotica* bark aqueous extract. More techniques

have to be executed for identifying the residing biologically dynamic principles within *A.nilotica* bark aqueous extract and their active isolation as well as purification for screening the pharmacological traits. This will undoubtedly speed up the process of developing novel drugs to combat antibiotic resistance and create health-restoring medications.

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