



Identification of Potential Inhibitors From *Urginea indica* Metabolites Against *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae* Receptors

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Synthetic pesticides are extensively used in agriculture to control pests and prevent yield loss. However, excessive use imposes a serious threat to human health, environment, and biodiversity; hence, certain pesticides have been abandoned from agricultural applications. Thus, there is a need to discover potential and eco-friendly pesticides for the effective management of phytopathogens. In current study, *Urginea indica* bulb extract was evaluated for potential antimicrobials and antioxidant phytochemicals. The methanol and aqueous extracts were prepared from the bulbs of *Urginea indica* and were evaluated for polyphenol contents, alkaloid, total antioxidant capacity, and iron chelating activity. Aqueous extract exhibited high phenol and flavonoid content, whereas the total antioxidant activity was higher in methanol extract. The iron chelating activity of both methanolic and aqueous extracts was approximately similar. The antioxidant activity of both methanolic and aqueous extracts was expressed in terms of IC₅₀ values for 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS), and nitric oxide (NO). The highest IC₅₀ value was observed for DPPH and the lowest for NO in both the extract. Further, fourier transform infrared spectroscopy (FTIR) was performed, which indicated the presence of several functional groups in the extract. In addition, 75 metabolites were recorded through gas chromatography–mass spectrometry (GC-MS), of which 23 were predicted to have antimicrobial activities. Consequently, metabolites were docked with D-alanine-D-alanine ligase A (DdIA) and mitogen-activated protein kinase 1 (MAPK1) of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Magnaporthe oryzae* (*M.oryzae*), respectively, to understand the possible mechanism of interaction between active metabolites and pathogen receptors. Docking study revealed that quinic acid, 3-caffeoyl has highest binding affinity for both DdIA and MAPK1 with respect to reference compound D-cycloserine and Trametinib. Thus, quinic acid, 3-caffeoyl could inhibit both DdIA and MAPK1-mediated signal transduction and, hence,

could be used as a promising natural inhibitor of DdIA and MAPK1 receptors. The above results indicate that *Urginea indica* could be a potential source of bioactive compounds and could be used as a potential source of natural pesticides to suppress phytopathogens.

Keywords: antimicrobial, antioxidant, metabolites, magnaporthe oryzae, pesticides, urginea indica, xanthomonas oryzae

INTRODUCTION

Crops are constantly exposed to various pests like bacteria, fungi, viruses, and insects, which greatly impair their growth, productivity, and quality. To protect crops from these pests, farmers routinely use synthetic chemicals. Although, synthetic chemicals have played vital roles in fulfilling developmental goals such as food security, livelihoods, and economic growth. However, inappropriate and indiscriminate use of pesticides causes undesirable health and environmental damage, and hence, certain chemicals have been abandoned from agriculture applications. In addition, the indelible use of synthetic chemicals directly/indirectly increases soil toxicity, kills beneficial microorganism, accelerates biodiversity loss, and drives the development of resistant pathogens (Nicolopoulou-Stamati et al., 2016; Shabana et al., 2017). Therefore, the damage associated with synthetic pesticides forced to ponder for a sustainable and ecological approach to reform agriculture through the implementation of naturally safe alternative pesticides. Interestingly, plant-derived natural compounds are less toxic, biodegradable, and eco-friendly (Fayaz et al., 2017; Campos et al., 2019). Notably, plants containing various bioactive compounds such as phenolics, flavonoids, coumarins, alkaloids, tannins, and terpenoids have been traditionally used to manage plant, human, and animal pathogens (Valli et al., 2012; Yuan et al., 2016; Anand et al., 2019). Pyrethrum (*Tanacetum cinerariifolium*), sweet wormwood (*Artemisia annua*), turmeric (*Curcuma longa*), eucalyptus (*Eucalyptus citriodora*), ginger (*Zingiber officinale*), wild basil (*Ocimum gratissimum*), lemongrass (*Cymbopogon citratus*), Neem (*Azadirachta indica*), etc., have been exploited as a promising source of natural pesticides/insecticides (Nisha et al., 2012; Alzohairy, 2016; Anyanwu and Okoye, 2017).

Rice, the staple food, faces two devastating infections termed as Bacterial Blight (BB) and rice blast and tents significant yield loss globally (Kumar et al., 2020; Jamaloddin et al., 2020). BB is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), whereas *Magnaporthe oryzae* (*M. oryzae*) is responsible for rice blast. Nowadays, researchers are targeting several receptor proteins of pathogens to understand the molecular mechanism of interaction between natural bioactive compounds and pathogen receptors. In particular, Eicosapentaenoic acid, Phytol, and Salicylic acid methyl ester of *Lantana camara* have shown greater affinity to peptide deformylase (PDF) of *Xoo* (Mansoori et al., 2020). Similarly, Copaene from *Thespesia lampas* has been reported to exhibit strong binding affinity with mitogen-activated protein kinase 1 (MAPK1) of *M.*

oryzae and sucrose hydrolase (SUH)–glucose complex of *Xanthomonas axonopodis* (Singh et al., 2021a). Recently, *Schefflera vinosa* molecules including 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane and 2-methoxy-5-methylthiophene) have been reported, which targets histone deacetylase and PDF of *M. oryzae* and *Xoo*, respectively (Singh et al., 2021b). In current study, D-alanine-D-alanine ligase A (DdIA) and MAPK1 of *Xoo* and *M. oryzae*, respectively, were selected for molecular docking studies with bioactive compounds acquired from gas chromatography–mass spectrometry (GC-MS). DdIA catalyzes the biosynthesis of d-alanyl-d-alanine, an essential precursor of peptidoglycan (Pederick et al., 2020). On the other side, MAPK1 regulates cell division through phosphorylation of protein residues (Xu et al., 2017).

Although few plants have been exploited, several plant species are yet to be exploited, and one such species is *Urginea indica* Kunth (Liliaceae family). It is commonly known as Jangli Pyaj (forest onion) and Bon Pollundu in India (Bashir et al., 2013) and has been used both in the pharmaceutical and agriculture sectors (Aswal et al., 2019). Extract of *U. indica* has been reported to contain several properties such as antioxidant, anticancer, antidiabetic, antiasthma, antigout, antiallergic, anti-dropsy, cardiac stimulant, wound healing, dyspepsia, and in treatment of various bacterial and plant pathogenic fungal diseases (Shenoy et al., 2006; Pandey and Gupta, 2014). Many phytochemicals like tannins, phenols, alkaloids, and flavonoids were reported in all parts of *U. indica*, whereas in bulb, steroids, resins, glycosides, carbohydrates, quinones, and saponins were reported (Chittoor et al., 2012; Pandey and Gupta, 2014). Since *U. indica* contains several medicinal compounds, a deeper investigation is needed for identification of bioactive compounds antagonists of phytopathogens such as *Xoo* and *M. oryzae*. Thus, the main objective of the current work was to systematically investigate the secondary metabolites from the crude extract of *U. indica* bulb and to examine the binding ability of bioactive compounds with *Xoo* and *M. oryzae* receptor proteins (DdIA and MAPK1). In addition, phytochemical profiling (qualitative and quantitative) was also performed to understand its antioxidant properties.

MATERIALS AND METHODS

Reagents

All chemicals and reagents used in these experiments were of analytical grade. Chemicals such as acetic acid (CH₃COOH), ammonium hydroxide (NH₄OH), methanol, gallic acid

monohydrate ($C_7H_6O_5 \cdot H_2O$), sodium carbonate (Na_2CO_3), quercetin dihydrate ($C_{15}H_{14}O_9$), ammonium molybdate ($(NH_4)_2MoO_4$), hydrochloric acid (HCl), chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS), L-ascorbic acid, sodium nitroprusside, orthophosphoric acid, naphthylene diamine dihydrochloride ferric chloride ninhydrin, ammonia, and sodium phosphate (monobasic) were procured from Hi-Media, India. Aluminum chloride ($AlCl_3$), sulfuric acid (H_2SO_4), and Folin-Ciocalteu reagent were purchased from Central Drug House (P) Ltd., India.

Plant Extract Preparation

Fresh *U. indica* bulb was collected from Amarkantak, Madhya Pradesh, India ($22^{\circ}40'N$ $81^{\circ}45'E/22.67^{\circ}N$ $81.75^{\circ}E/22.67; 81.75$). Afterward, samples were cleaned with tap and distilled water. Subsequently, samples were dried at room temperature (RT) and crushed to a fine powder with a mechanical grinder. The powdered materials (10 g) were macerated with 100 ml of 80% methanol and 100 ml of water for three days using a magnetic stirrer. The residues were re-macerated for three times to completely extract the phytoconstituent. The extract was then filtered through the Whatman No. 1 filter paper and concentrated to dry mass at RT. The dried residues were harvested and transferred into airtight bottles and were stored at $4^{\circ}C$ in a refrigerator until use. The dried extracts were dissolved in methanol and water as per the requirements of the experiment. The yield of crude extract was calculated and expressed in percentages as: $Yield\% = \text{weight of the dry extract} \times 100 / \text{weight of the dry plant material}$ (Singh et al., 2021a).

Qualitative Phytochemical Screening

Preliminary phytochemical qualitative screening of *U. indica* methanolic and aqueous extract (ME and AE) was carried out to determine the existence of biologically active compounds/secondary metabolites like saponins, tannins, flavonoid, and steroids using as method described by Harborne (1998). Similarly, the presence/absence of alkaloids, coumarins, terpenoids, glycosides, phenols, quinones, amino acids, cardiac glycosides, phlobatannins, anthocyanins, and anthraquinones was unveiled using the Roghini and Vijayalakshmi (2018) method.

Quantitative Phytochemical Screening

Total Phenol Content

The total phenol content (TPC) was estimated according to the Folin-Ciocalteu method through spectrophotometer with slight modifications (Singleton et al., 1999). First, methanolic and aqueous solution was made in a concentration of 300 $\mu\text{g/ml}$ and then blended with 10% Folin-Ciocalteu reagent (2.5 ml) followed by 7.5% Na_2CO_3 (2.5 ml). Whole solution was vortexed for 2 min and dark-incubated at RT for 45 minutes. Subsequently, absorbance was read at 750 nm. Standard calibration curve was made using gallic acid (20–100 $\mu\text{g/ml}$). The TPC was represented as mg of gallic acid equivalents per gram of dry weight (mg GAE/g dw) and was calculated as mean value \pm SD ($n = 3$).

Total Flavonoids Content (TFC)

The total flavonoids content (TFC) of extract was estimated through the aluminum chloride ($AlCl_3$) colorimetric method (Chang et al., 2002; Kumar et al., 2011). Briefly, reaction solution was made by mixing 2.0 ml of diluted extract/quercetin, $AlCl_3$ (0.1 ml, 10%), and CH_3CO_2K (0.1 ml, 0.1 mM). Afterward, absorbance was acquired at 415 nm after 2 min of vortexing and 30 min of dark incubation at RT. Quercetin was used to prepare the standard curve (10–60 $\mu\text{g/ml}$), and, finally, TFC in extract was represented as mg of quercetin equivalents per gram of dry weight (mg QE/g dw) and was calculated as mean value \pm SD ($n = 3$).

Total Antioxidants Activity

The total antioxidants activity (TAA) in ME and AE was evaluated through phosphomolybdenum method (Govindarajan et al., 2003; Kumar et al., 2013) with minor amendments. Altogether, a 0.2-ml extract was amended with 1.8 ml of phosphomolybdenum reagent [0.6M H_2SO_4 , 28mM Na_3PO_4 , and 4mM $(NH_4)_6Mo_7O_{24}$]. After mixing for 2 min, the solution was incubated for 90 min at $95^{\circ}C$ in a water bath. Finally, mixture was cooled at RT, and absorbance was taken at 695 nm through a spectrophotometer. Ascorbic acid (20–100 $\mu\text{g/ml}$) was taken as the standard, and result was represented as milligrams of ascorbic acid equivalents per gram of dry weight (mg AAE/g dw) and calculated as mean value \pm SD ($n = 3$).

Total Alkaloid Content

The total alkaloid content (TAC) in extract was assessed following the standard method (Harborne, 1973). Plant powder (2.5 gm) was mixed with 200 ml of acetic acid in methanol (10%). Afterward, the mixture was incubated for 4 h at RT. Then, until final precipitation, a few drops of concentrated ammonium hydroxide (NH_4OH) were added. The precipitates were then rinsed in 25 ml of 0.1 M NH_4OH . Finally, the supernatant was air-dried, and the result was expressed in percentage using the following calculation: $\text{Percent alkaloid} = \text{weight of alkaloid} \times 100 / \text{weight of sample}$.

Iron Chelating Activity

The capacity of *U. indica* crude extract to bind iron (I) was determined using the procedure by Dinis et al. (1994). The presence of ferrous ions is indicated by the development of a red ferrous iron-ferrozine complex, which is evaluated by measuring absorbance at 562 nm. In a separate test tube, 2.0-ml extract and standard (Na_2EDTA) at working concentrations of 20, 40, and 100 $\mu\text{g/ml}$ were combined with 400 μl of ferrous sulfate (2.0 mM). The reaction was initiated by the addition of ferrozine into the mixture. Finally, the absorbance of the sample was measured at 562 nm, and the percent inhibition was determined using the following equation: $\text{Scavenging effect \%} = (\text{control absorbance} - \text{sample absorbance}) \times 100 / \text{control absorbance}$.

Nitric Oxide Radical Scavenging Activity

The capacity of the crude plant extract to scavenge the nitric oxide (NO) free radical was assessed through a conventional

approach (Sreejayan Rao, 1997) with slight modifications. The reaction was started by adding 200 μ l of plant crude extract at various concentrations in separate test tubes, followed by 80 μ l of sodium nitroprusside, and then incubated for 15 min under light conditions. After 15 min, 1.0 ml of Griess reagent was added to this combination, and all the tubes were incubated for 45 min at 30°C under light conditions. To halt the process, 40 μ l of 0.1% naphthylene diamine dihydrochloride in 2.5% H₃PO₄ was added. Using double-distilled water (DDW), the final volume was increased to 2.0 ml, and the absorbance was measured at $\lambda_{\text{max}} = 540$ nm. The scavenging activity was determined in terms of NO radicals scavenged using the following equation: Scavenging effect % = (control absorbance – sample absorbance) \times 100/control absorbance

2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity

The extract's free radical scavenging activity was determined using conventional DPPH tests (Bursal and Gülçin, 2011; Kumar et al., 2011; Kumar et al., 2013). The solution was made by dissolving 4.0 mg of DPPH in 100 ml of methanol, and absorbance was measured at $\lambda_{\text{max}} = 516$ nm with a spectrophotometer. This solution was combined with 300 μ l of standard (Ascorbic acid) or extract at various concentrations (20–220 μ g/ml). After shaking, the reaction mixture was incubated in the dark for 15 min at RT. The final absorbance was taken at $\lambda_{\text{max}} = 515$ nm. The scavenging activity was determined in terms of the DPPH radical scavenged using the following equation: Scavenging effect % = (control absorbance – sample absorbance) \times 100/control absorbance.

2,2'-Azino-Bis-3-Ethylbenzotiazolin-6-Sulfonic Acid Radical Scavenging Activity

The capacity of a crude plant extract to scavenge ABTS radicals was assessed through a conventional method (Re et al., 1999). The mechanism of the reaction includes the decolorization of ABTS^{•+} radicals *via* the electron donating activity of plant extract antioxidants. ABTS solution (7.0 mM) was produced with DDW and combined in a 1:1 ratio with 2.45 mM K₂S₂O₈ solution. Following that, a 24- to 48-h dark incubation at RT was performed. The ABTS solution was then diluted with methanol, and the absorbance was adjusted at 0.700 \pm 0.02 at $\lambda_{\text{max}} = 745$ nm. A mixture of extract/standard concentrations ranging from 20 to 220 μ g/ml (300 μ l) was combined with 2.5 ml of solution. After shaking, the entire reaction mixture was incubated in the dark at RT for 15 min. Finally, absorbance was measured at $\lambda_{\text{max}} = 745$ nm. The scavenging activity was calculated in terms of the percent inhibition of ABTS^{•+} using the same equation as the percent inhibition of DPPH radical scavenging activity.

Fourier Transform Infrared Spectroscopy Spectral Analysis

The fourier transform infrared spectroscopy (FTIR) spectra of dried *U. indica* extract were measured at RT, utilizing attenuated total reflectance (ATR) and internal reflection element formed of

diamond using ATR equipment Thermo Nicolet iS5 (Thermo Scientific, Madison, WI, USA). This FTIR system is built using a KBr beam splitter and a deuterated triglycine sulfate detector, and it is linked to a Windows-based system running OMNIC software (OMNICTM Series Software; thermofisher.com). All measurements were collected at a resolution of 4 cm⁻¹ in the spectral band of 4,000 to 500 cm⁻¹. The spectra of the samples were subtracted from the spectra of the air. At each point, the spectra were logged as an absorbance value. During measurement, the ATR crystal surface was cleaned with 70% ethanol on a regular basis.

Gas Chromatography–Mass Spectrometry Analysis

To determine the presence of metabolites, gas chromatography–mass spectrometry (GC-MS) was performed according to Sharma et al. (2021) and Kilambi et al. (2021) (a modified method of Roessner et al., 2000). Fraction of metabolite was prepared by mixing 50 mg of dry powder with 100% chilled methanol (0.95 ml) containing ribitol as internal standard succeeded by incubation at 70°C for 15 min with continuous shaking. After incubation, 0.95 ml of chilled water was added and at 2,500g centrifugation was performed for 15 min at 4°C. The supernatant was relocated to a new centrifuge tube and allowed to dry for 120 min. Further, derivatization of obtained residue was done by mixing methoxamine (65 μ l) containing pyridine at 37°C for 90 min following the incubation with 65 μ l of N-methyl-N-(trimethylsilyl) trifluoroacetamine and FAME mix (20 μ l) at 37°C for 30 min. The LECO-PEGASUS GCXGC-TOF-MS system (LECO Corporation, USA) was used to analyze the derivatized sample, which was equipped with a 30-m Rxi-5ms column with 0.25-mm internal diameter and 0.25- μ m film thickness (Restek, USA). Sources of injection temperature, interference, and ion temperature were set to 240°C, 225°C, and 200°C, respectively. The following steps were persuaded for sample chromatographic separation: isothermal heating (70°C) for 5 min, succeeded by a 5°C min⁻¹ oven temperature ramp to 290°C and 5 min of heating at 290°C. Helium gas was utilized as carrier, and their flow rate was attuned to 1.4 ml/min. Finally, 1 μ l of sample was administered in split less mode for investigation, and the range of scan mass was set to 70 to 600 at 2 scans/s.

Metabolite Identifications

Metabolite identification was performed according to Sharma et al. (2021). Data files were generated as netCDF by ChromaTOF software 299 4.50.8.0 chromatography version (LECO Corporation, USA) and examined using MetAlign 3.0 software (www.metalign.nl) (Lommen and Kools, 2012). Several parameters were acquainted such as signal-to-noise ratio of \geq 2, baseline correction, noise estimation, and mass peak identification (ion-wise mass alignment). Data reduction and mass extraction of compounds were performed through MSClust analysis (Tikunov et al., 2012). Finally, the files obtained were exported to the NIST MS Search v2.2 program to identify compounds using the NIST (National Institute of Standards

and Technology) library and the GOLM metabolome database library (Hummel et al., 2010). All the metabolites were quantified by normalization with ribitol (internal standard).

Molecular Docking

Ligand and Protein Preparation

As mentioned, 66 ligands were acquired from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in Protein Data Bank (PDB) format and were converted into PDBQT format for docking analysis. Three-dimensional structure of DDdIA (PDB ID: 4ME6) and MAPK1 (PDB ID: 5z33) was acquired from PDB (<https://www.rcsb.org/>) in Docking Parameter Format (PDB) format. Heteroatoms including water and others molecules were eliminated using BIOVIA Discovery Studio. Missing atoms of amino acid chain were corrected with Swiss PDB viewer. Active sites of DdIA and MAPK1 were detected from Computed Atlas of Surface Topography of protein (CASTp) online server (Tian et al., 2018) and discovery studio, respectively.

Docking Analysis

Autodock 4.2.6 was used for analysis together with accompanying software, viz., Python 3.9.6 and MGLTools 1.5.6. Protein ligand docking was accomplished by binding one ligand at a time to the protein receptor (Morris et al., 2009). The processed protein was then imported into an Autodock workspace for the insertion of polar hydrogen atoms, as well as Kollman and Gasteiger charges (Ravindranath et al., 2015). Similarly, ligands were incorporated into the same place to define its torsion tree by choosing a root and a rotatable number of bonds. Both the protein and ligands were saved in PDBQT format for further process.

Preparation of Grid Parameter and Autodocking

Grid parameters were defined for both the proteins to navigate the active site for proper interaction of ligands. For DdIA, the center grid box values were set to $x = 2.481$, $y = 12.094$, and $z = 34.667$ and for MAPK1, $x = 51.07$, $y = 33.07$, and $z = 28.2$. Grid points for both the receptor proteins in the x , y , and z dimensions were set to 68, 62, and 68, respectively. Autogrid was successfully executed, and then, genetic algorithm was set to the following parameters: 1) numbers of GA runs: 50; 2) population size: 300; 3) energy evaluations number: 2.5 million; and 4) generations number: 27,000. The Lamarckian genetic process was used subsequently, and the output file was stored in DPF. Finally, the program was launched for docking, and output of the final result was saved as docking log file. Binding energy, inhibition constant, and ligand efficiency were calculated, and the complex had lower binding energy that was visualized in discovery studio for 2D and 3D evaluation.

Absorption, Distribution, Metabolism, Elimination, and Toxicity Property Prediction

Each compound which had good binding affinity with proteins was subjected to absorption, distribution, metabolism,

elimination, and toxicity (ADMET) property prediction. It is a computer program that calculates the pharmacokinetics and drug-like properties of substances based on their chemical structure. The smiley format was introduced at interface of SwissADME website (<http://www.swissadme.ch/>), and program was run to generate ADMET properties/parameters.

STATISTICAL ANALYSIS

Both ME and AE were taken in triplicates for all biochemical assays. The results were denoted as mean \pm standard deviation (SD) ($n = 3$). GC-MS results were expressed as an average of five replicates. Two-way analysis of variance (ANOVA) was executed to assess the variation among TPC, TFC, and TAA of ME and AE. The interrelations among DPPH, ABTS, NO radical scavenging assay, and iron chelating activity were studied using the Pearson correlation coefficient test. OriginPro 2016 and GraphPad Prism 8 were used to create the statistical analysis and graph preparation.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis of Methanolic and Aqueous Extract of *U. indica*

In both ME and AE, preliminary phytochemical screening discovered the occurrence of alkaloids, steroids, flavonoids, quinones, and coumarins (**Table 1**). Saponins and terpenoids were found in only AE, whereas amino acids were only detected in ME. Each detected phytochemical has shown several medicinal properties against different microbial diseases. Alkaloids are a diverse group of secondary metabolites that have inspired the development of several antibacterial compounds such as ascididemin, eudistomin, squalamine, and clausenol against Gram-positive and Gram-negative bacteria (Cushnie et al., 2014). Predominantly, flavonoids have been used as an antifungal agent against *Candida albicans*, *Trichophyton rubrum*, and *Aspergillus flavus* (Lee and Chee, 2010; Mohotti et al., 2020; Quiroga et al., 2009; Bitencourt et al., 2013). Terpenoids and their derivatives usually affect oxygen uptake and oxidative phosphorylation, which are essential to microbial survival (Griffin et al., 1999). Plant-derived quinones have been used as an anticancer and antibacterial agent (Goel et al., 2020). Several studies have reported that coumarins exhibited potent antibacterial activity especially against Gram-negative bacteria through their cell membrane damage (Céspedes et al., 2006; Saleem et al., 2010; Yu, 2015). Steroids have been used as cationic forms for bacterial membrane targets based on specificity (Savage et al., 2002). In addition, there are several reports that suggest that consuming natural food containing phenolics compounds decreases the risk of health disorder due to the antioxidant nature of phenolics (Surh, 2002). Thus, the present investigation suggests that *U. indica* phytochemicals could be a promising agent, particularly against plant pathogens and in nutraceuticals.

TABLE 1 | Qualitative analysis of phytochemicals presents in methanolic and aqueous extract of *U. indica* bulb.

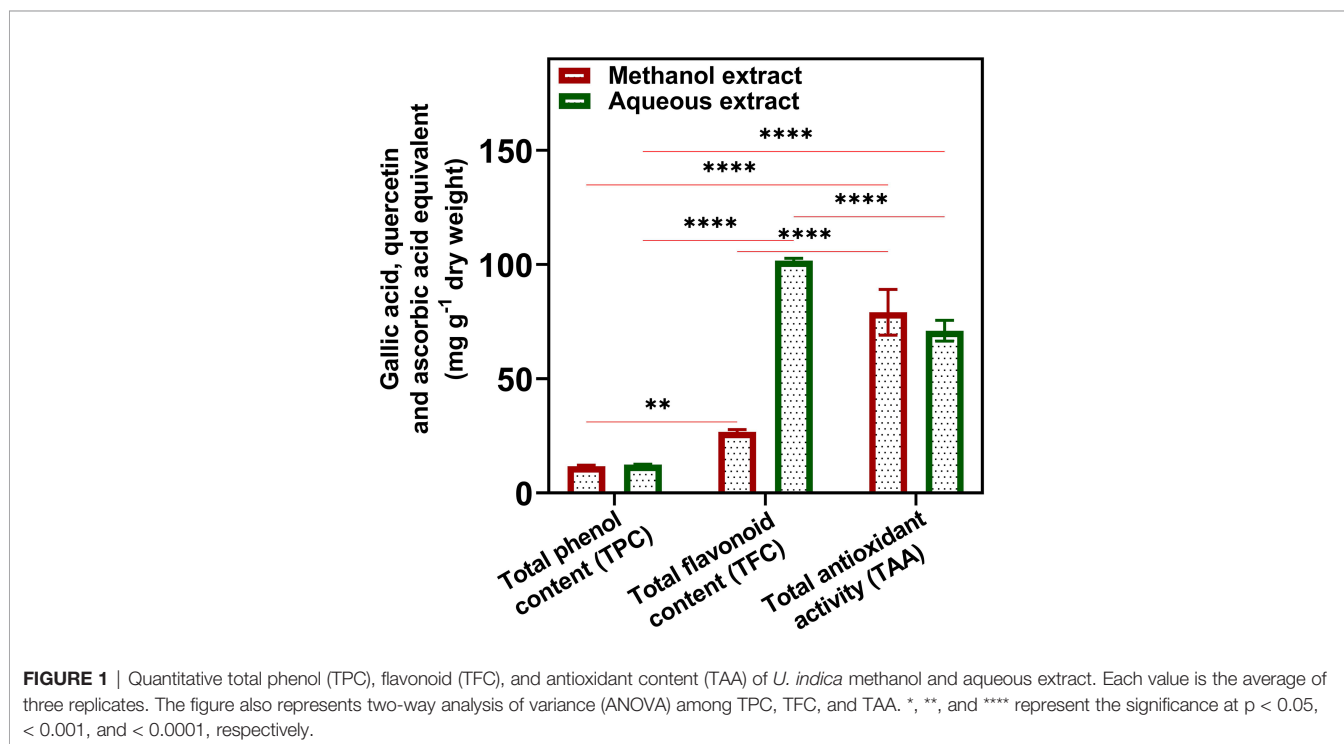
| S. No. | Phytochemicals | Methanol Extract | Aqueous Extract |
|--------|--------------------|------------------|-----------------|
| 1. | Alkaloids | + | + |
| 2. | Saponins | - | +++ |
| 3. | Tannins | - | - |
| 4. | Flavonoids | ++ | ++ |
| 5. | Terpenoids | - | +++ |
| 6. | Glycosides | - | - |
| 7. | Phenols | - | - |
| 8. | Quinones | +++ | ++ |
| 9. | Cardiac glycosides | - | - |
| 10. | Amino acid | +++ | - |
| 11. | Coumarin | + | ++ |
| 12. | Phlobatannins | - | - |
| 13. | Antraquinones | - | - |
| 14. | Steroids | ++ | +++ |

+++, highly present; ++, moderately present; +, least present; -, absent.

Determination of Total Alkaloids, Phenols, Flavonoids, and Antioxidants Contents

TAC was $2.4 \pm 0.42\%$ in *U. indica* powder. TPC, TFC, and TAA calculations were based on the equation obtained from gallic acid, quercetin, and ascorbic acid, respectively (**Supplementary Figure 1**). The TPC in both ME and AE was slightly similar in terms of mg GAE/g dw. The ME demonstrated 11.73 ± 0.46 mg GAE/g dw, whereas, in aqueous extract, GAE was found to be 12.40 ± 0.20 mg/g dw (**Figure 1**). The AE exhibited higher amounts of flavonoid content (101.78 ± 0.98 mg QE/g dw) than ME (26.81 ± 0.98 mg/g dw). Further, the TAA was found to be higher in ME (79.09 ± 10.00) as compared to AE (71.03 ± 4.50) in terms of mg AAE/g dw. In the present study, flavonoid content was higher in AE among all tested phytochemicals. In addition, it

found that water was a better solvent for the extraction of phenols and flavonoids from *U. indica*. Both TPC and TAA were similar in ME and AE, suggesting the extraction of polar nature of compounds due to polar nature of phenols (Garcia-Salas et al., 2010). Previous studies also suggest a correlation between total phenol and antioxidants (Kumar et al., 2014). We have also studied the interaction between TPC, TFC, and TAA of ME and AE of *U. indica* using two-way ANOVA (**Figure 1**), which demonstrated that TPC and TFC of ME are significantly different at a level of $P < 0.05$, whereas TAA of ME also exhibited highly dissimilarities with both TPC and TFC at a level of $P < 0.0001$. However, TPC and TFC of AE have significantly different at a level of $P < 0.0001$, whereas TAA of AE has variation with TPC at a level of $P < 0.0001$ and with TFC at a level of $P < 0.0001$.



The above result justifies the significant contribution of flavonoids and phenols to plants' phosphomolybdate scavenging-related total antioxidant activity. Phenols lose H^+ ion to produce phenolate ion, which subsequently reacts with Folin–Ciocalteu reagent to reduce them (Fernandes et al., 2012). As phenolics and many flavonoids contain polar hydroxyl groups, their higher extraction with methanol and water is quite reasonable (Ahmed et al., 2015). High phenolic content in plants makes them a potential source for various therapeutic uses for the treatment of chronic diseases related to microbial infections (Petti and Scully, 2009). Likewise, flavonoid has a wide range of biological functions and is used as an anti-cancer, anti-inflammatory, anti-allergic, anti-thrombotic, anti-tumor inhibitor, and anti-viral agent (Trease and Evans, 2002; Aggarwal and Shishodia, 2006). The presence of phenolics and flavonoids in *U. indica* makes them a potent antimicrobial agent as it demonstrated microbial inhibitory effects previously (Lim et al., 2007; Jarial et al., 2018; Dzotam et al., 2018). Hence, results obtained demonstrate that *U. indica* would be of particular interest for further research.

Iron Chelating Activity Determination

The Fe^{2+} ion chelating activity of *U. indica* ME and AE was determined at concentrations of 20, 60, and 100 $\mu\text{g/ml}$. At a concentration of 100 $\mu\text{g/ml}$, the result obtained in terms of percent inhibition of Fe^{2+} ion was determined to be 85.22 ± 0.513 for ME and 85.39 ± 3.69 for AE. As a positive control, Na_2EDTA demonstrated chelating activity of $98.68 \pm 0.425\%$ at the maximum concentration of 100 $\mu\text{g/ml}$ (Figure 2A). As a result, the chelating activity of standard is greater than that of ME and

AE. However, ME's IC_{50} value is almost similar to AE's, indicating that both ME and AE have equal iron chelating potential (Table 2). Previous studies have reported that the iron chelators like caffeic acid act like antioxidants and reactive oxygen species scavengers. Interestingly, iron overload is neutralized by chelation therapy in case of thalassemia and anemia, and synthetic chelators are also used to control oxidative damage, which have shown negative impact on health as well (Ebrahimzadeh et al., 2008). Hence, plant-derived molecules could be used for chelation as an alternative to synthetic chelators.

NO Radical Scavenging Activity Determination

The reactive capability of the NO radical was estimated by measuring the decrease in absorbance at $\lambda_{max} = 546 \text{ nm}$ caused by antioxidant activation. The antioxidant activity values of *U. indica* ME, AE, and standard ascorbic acid at 20–220 $\mu\text{g/ml}$ were 4.96 ± 2.96 to 31.77 ± 2.41 , 11.38 ± 2.81 to 41.91 ± 2.96 , and 48.95 ± 0.17 to 84.47 ± 0.31 , respectively, as shown in Figure 2B. It clearly shows that *U. indica* has a dose-dependent NO scavenging action, with an IC_{50} value of $323.44 \pm 16.31 \mu\text{g/ml}$ for ME and $275.75 \pm 11.15 \mu\text{g/ml}$ for AE, whereas ascorbic acid has an IC_{50} value of $74.57 \pm 0.611 \mu\text{g/ml}$ (Table 2). The Pearson correlation coefficient values of NO radical scavenging activity of plant extract with TAA and iron chelating activity suggest that both are positively correlated with correlation coefficient values of 0.32 and 0.42, respectively. NO overproduction has been related to a variety of chronic diseases, including carcinogenesis and arteriosclerosis. It is poisonous, and it combines with other free radical molecules

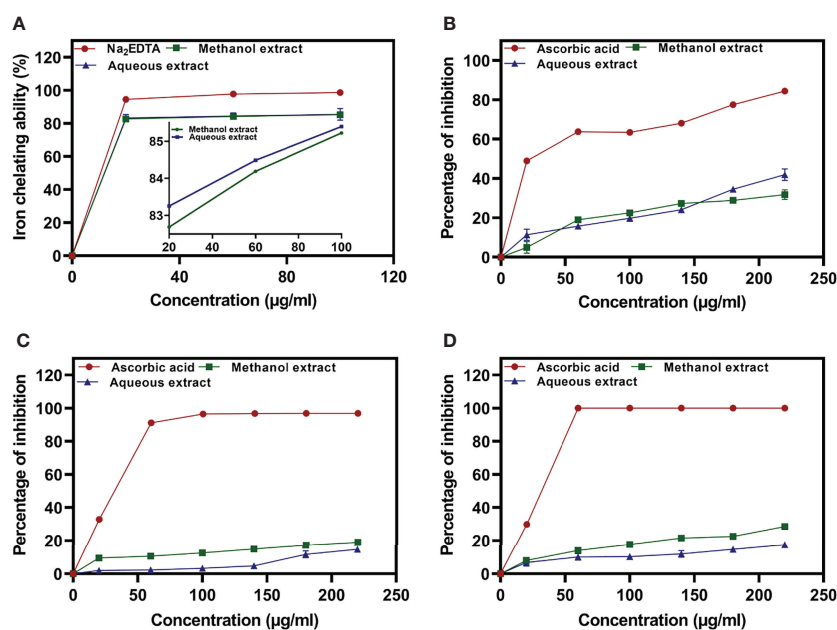


FIGURE 2 | Iron chelating activity of methanolic and aqueous extract of *U. indica*, along with Na_2EDTA standard (A), percentage inhibition of nitric oxide radicals (B), DPPH radicals (C), and ABTS radicals (D) via methanolic and aqueous of *U. indica* along with ascorbic acid standard.

TABLE 2 | IC₅₀ value of methanolic and aqueous extract of *U. indica*.

| Sample | DPPH | IC ₅₀ µg/ml ABTS | Nitric Oxide | Iron Chelating |
|----------------------|----------------|--------------------------------|----------------|----------------|
| ME | 648.16 ± 27.65 | 403.55 ± 7.91 | 323.44 ± 16.31 | 25.12 ± 0.54 |
| AE | 789.52 ± 86.64 | 722.58 ± 15.08 | 275.75 ± 11.15 | 24.84 ± 1.89 |
| Ascorbic acid | 43.02 ± 0.895 | 38.53 ± 0.11 | 74.57 ± 0.611 | 17.55 ± 3.78 |
| Na ₂ EDTA | - | - | - | - |

to increase toxicity (Valko et al., 2007). Because *U. indica* extract has NO scavenging activity, it may be effective in reducing free radical-related damage and illnesses.

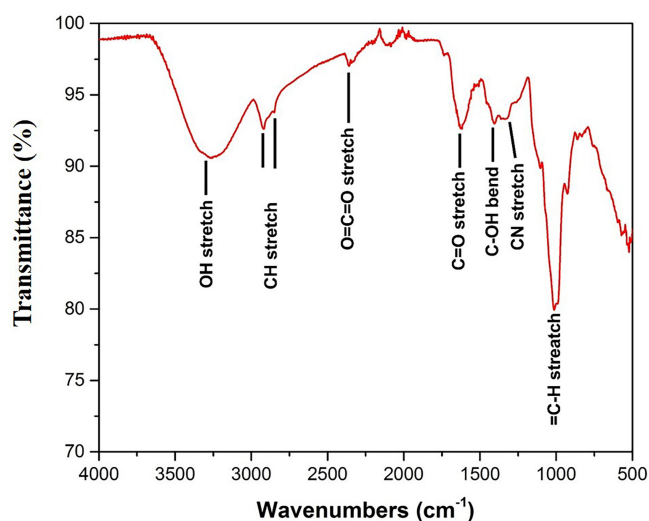
DPPH Radical Scavenging Activity Determination

Concentration-dependent increases in DPPH radical scavenging capacity of *U. indica* extract are noted as depicted in **Figure 2C**. The ME and AE of *U. indica* had the most potent scavenging effect, with an IC₅₀ value of 648.16 ± 27.65 and 789.52 ± 86.64 µg/ml, respectively. When the extract's scavenging capability was compared to the standard (ascorbic acid), the IC₅₀ value was 43.02 ± 0.895. (**Table 2**). The methanolic extract's scavenging activity in terms of percent inhibition varies from 9.55 ± 1.37% to 18.99 ± 1.22%, whereas aqueous extract's scavenging activity in terms of percent inhibition varies from 1.97 ± 0.12% to 14.72 ± 0.81%, and the standard ranges from 32.82 ± 0.99% to 96.83 ± 0.063% at concentrations ranging from 20 to 220 µg/ml. In terms of low IC₅₀ value, a comparative investigation has shown that ME of *U. indica* had greater antioxidant activity than AE (**Table 2**). Pearson correlation result also suggests that DPPH radical scavenging activity has positive correlation with ABTS radical scavenging activity (with correlation coefficient value 0.81) followed by TFC (with correlation coefficient value 0.79) and TPC (with correlation coefficient value 0.55)

(**Supplementary Figure 2**). The DPPH test is an appropriate and widely used technique for determining the free radical scavenging capability of plant extracts. Phenolic compounds contain hydroxyl groups that are responsible for radical scavenging effects due to their redox properties (Evans et al., 1997). A study indicated that the natural antioxidants derived from plants have been reported as an antimicrobial agent with possible mechanisms of microbial enzyme inhibition, substrate deprivation, and protein regulation (Aminov, 2010). Hence, high free radicals' activity in the current study could be attributed to high flavonoid and phenolic content in plant extract, which may contribute to *Xoo* and *M. oryzae* inhibition. Interestingly, extract having both antioxidant and DPPH radical scavenging ability donates hydrogen to free radicals that potentially inhibit the progression of lipid peroxidation (Bamforth et al., 1993).

ABTS Radical Scavenging Activity Determination

The interaction of ABTS and K₂S₂O₈ produces ABTS cation. The hydrogen/electron donating abilities of plant extract were demonstrated by assessing the scavenging potential of ABTS cations using *U. indica* ME and AE. *U. indica* crude extract demonstrated concentration-dependent ABTS radical scavenging capacity (20–220 µg/ml), as shown in **Figure 2D**. The linear response curves were also produced, and the IC₅₀

**FIGURE 3** | FTIR spectra of *U. indica* extract. Extract was put on diamond crystal pedestal of ATR and readings were taken from 500 to 4,000 cm⁻¹.

value for ME and AE was determined to be 403.55 ± 7.91 $\mu\text{g/ml}$ and 722.58 ± 15.08 $\mu\text{g/ml}$, respectively, and 38.53 ± 0.11 $\mu\text{g/ml}$ for ascorbic acid. ME has a stronger ABTS scavenging activity than AE (Table 2). This experiment also shows that *U. indica* has free radical scavenging action and, hence, might be employed as an active component in medicines and free radical-related therapies. The correlation of ABTS radical scavenging capacity exhibited a positive correlation with TFC (with correlation coefficient value of 1.0) and TPC (with correlation coefficient value of 0.73) (Supplementary Figure 2). Significant correlation of ABTS with TFC and TPC could be a reason for scavenging potential of *U. indica*. Actually, the cation radical scavenging activity of ABTS indicates the donating capability of hydrogen. Previous reports suggest that phenolics of high molecular weight possess a greater ability for free radical (ABTS⁺) quenching (Hagerman et al., 1998). This scavenging feature leads to prevent oxidation of lipids through chain termination reaction and, hence, could be incorporated as an important nutraceutical when consumed with another nutrient as well.

Functional Group Analysis of *U. indica* Extract by FTIR Spectrum Analysis

The FTIR study was performed to bring out the fingerprints of the chemical constituents of the *U. indica* extract (Figure 3). It is a sophisticated approach for detecting functional groups in extracts based on the peak values acquired from the FTIR spectrum. The absorbance bands were analyzed in the region at a resolution between 500 and 4,000 cm^{-1} . Importantly, 3,269 (OH- stretching), 2,916 (CH- stretching), 2,846 (CH- stretching), 2,357 (O=C=O stretching), 1,732 (C=O stretching), 1,403 (C-OH bending), 1,326 (CN components), and 1,021 (=C-H stretching) cm^{-1} were assigned to several possible functional group such as alkanes, alkenes, amines, aromatic compounds/ carboxylic acids, aliphatic ketones, alcohols, and phenol related compounds. These functional groups may be responsible for the crude extract's antioxidant action as it is an integral part of various secondary metabolites such as phenols and flavonoids (Poojary et al., 2015). Table 3 summarizes the peak value, anticipated functional groups, and type of bond. Previous research indicates that functional group like aldehyde and phenolics participate in different biological functions, such as antiseptics, insecticides, fungicides, and plant immunity (Rao, 2012; Chandra, 2019; Singh et al., 2021b). Hence, it can be assumed that the *U. indica* antimicrobial property could have been contributed by such a functional group. As a result, its

therapeutic usage in Unani medicine appears to be validated. However, it has been reported that FTIR alone could not detect all the functional group of active constituents present in the crude extract, and hence, GC-MS study was also performed.

Metabolite Identification in Extract by GC-MS Based Metabolomics

U. indica extract was administered to GC-MS for detection of metabolites. Analysis was performed for five replicates of the sample for accuracy. A total of 75 compounds were detected in GC-MS metabolomics, which was categorized under different groups such as organic acids, amino acids, monoamines, sugars, sugar alcohols, sugar acids, fatty acids, and alcohols (Supplementary Table 1). In terms of relative levels, higher amounts of sugars were detected, viz., sorbose (32.78 ± 3.07), fructose (24.12 ± 1.96), and sucrose (21.80 ± 2.66), followed by organic acids such as malic acid (20.56 ± 1.27) and phosphoric acid (14.26 ± 1.21). Some metabolites were also confined to phenolic compounds, which are quinic acid, 3-caffeoyl quinic acid, tyrosol, pyrogallol, resorcinol, hydroquinone, and caffeic acid. Phenolic compounds detection may confirm the TPC of *U. indica* extract. Many studies have supported the phenol as an antimicrobial agent both experimentally and theoretically (Alves et al., 2013; Vidhya et al., 2020; Aliye et al., 2021). *In silico* analysis revealed the potential of phenol including diosmin, morin, and chlorogenic acid, against bacterial urease (Kataria and Khatkar, 2019). In addition, phenolic compounds exhibited *Staphylococcus aureus* membrane damage under *in vitro* conditions (Miklasińska-Majdanik et al., 2018). Perez-Castillo et al. (2020) reported that cinnamic acid and benzoic acid act against *Candida albicans*. Interestingly, 23 compounds identified in *U. indica* have previously reported antimicrobial activity against different human and plant pathogens (Table 4). Thus, the detection of an array of phytochemicals signifies the endowed medicinal properties of *U. indica*. These findings may aid in a more rational evaluation of the multipurpose usage of *U. indica* plant as a potential and natural source of antimicrobial compounds.

Validation of Antimicrobial Property of Plant Extract Through Molecular Docking

To examine binding affinity, molecular docking was performed for 66 compounds (excluded amino acids) against DdlA and MAPK1 of *Xoo* and *M. oryzae*, respectively. Virtual screening technologies are often and widely used as a strategy for discovering novel ligands for protein structures. Top three compounds were selected which had maximum binding affinity with both the proteins (Table 5).

TABLE 3 | FTIR analysis of probable functional group present in *U. indica* extract.

| Wavenumber (cm^{-1}) | Appearance | Types of Bond | Possible Functional Group |
|---------------------------------|------------|------------------|---------------------------|
| 3,296.03 | Broad | OH- stretching | Phenol, carboxylic acids |
| 2,916.00 | Sharp | CH- stretching | Alkane |
| 2,846 | Medium | CH- stretching | Alkane |
| 2,357 | Weak | O=C=O stretching | Carbone dioxide |
| 1,732 | Sharp | C=O stretching | Aliphatic ketone |
| 1,403 | Medium | C-OH bending | Alcohols |
| 1,326 | Medium | CN stretching | Amine |
| 1,012 | Sharp | =C-H stretching | Alkene |

TABLE 4 | Antimicrobial metabolites identified in GC-MS analysis of *U. indica* bulb extract.

| S. No. | Compound | Compound Nature | Biological Activity | Reference |
|--------|-------------------------|-----------------|---|--|
| 1. | Lactic acid | Organic acid | Antimicrobial activity against <i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i> , and <i>Rhodotorula</i> sp. | Stanojević-Nikolić et al., 2016 |
| 2. | Glycolic acid | Organic acid | Antibacterial activity against <i>Pseudomonas fluorescens</i> as membrane oxidative agent | Fernandes et al., 2020 |
| 3. | Phosphoric acid | Organic acid | Bactericidal activity against <i>Enterococcus faecalis</i> and antimicrobial activity against <i>Parvimonas micra</i> , <i>Porphyromonas gingivalis</i> , and <i>Prevotella nigrescens</i> | Arias-Moliz et al., 2008; Prado et al., 2015 |
| 4. | Succinic acid | Organic acid | Antibacterial activity against <i>E. coli</i> . | Kumar et al., 2018 |
| 5. | Fumaric acid | Organic acid | Antibacterial activity against <i>E. coli</i> and <i>L. monocytogenes</i> through disruption of their glutamate decarboxylase (GAD) system under acetic condition. | Barnes et al., 2020 |
| 6. | Malic acid | Organic acid | Antimicrobial activity against <i>L. monocytogenes</i> , <i>Salmonella enteritidis</i> , <i>E. coli</i> , and <i>S. gaminara</i> | Rosa et al., 2009; Eswaranandam et al., 2004 |
| 7. | 3-Hydroxybenzoic acid | Organic acid | Antimicrobial activity against <i>Gonoderma boninense</i> | Chong et al., 2009 |
| 8. | Shikimic acid | Organic acid | Antibacterial activity against <i>E. coli</i> and <i>Staphylococcus aureus</i> | Tripathi et al., 2015 |
| 9. | Quinic acid | Organic acid | Inhibitory effect on biofilm formation formed by <i>Staphylococcus aureus</i> through the suppression and activation of <i>sarA</i> and <i>agrA</i> gene, respectively. | Bai et al., 2019 |
| 10. | Quinic acid, 3-caffeoyl | Organic acid | Antibacterial activity against pathogenic bacteria such as <i>Shigella dysenteriae</i> and <i>Streptococcus pneumoniae</i> through their outer plasma membrane permeability; antibiofilm activity against <i>Stenotrophomonas maltophilia</i> | Lou et al., 2011; Karunanidhi et al., 2013 |
| 11. | 4-Coumaric acid | Organic acid | Antibacterial activity against <i>Escherichia coli</i> ; inhibitory effect on <i>Shigella dysenteriae</i> through their membrane permeability | Alves et al., 2013; Lou et al., 2012 |
| 12. | Caffeic acid | Organic acid | Antibacterial activity with capsulation of Beta cyclodextrins against <i>Staphylococcus aureus</i> | Pinho et al., 2015 |
| 13. | Arabinofuranose | Sugar | Conjugates of arabinofuranose and diterpenoid isosteviol exhibited antituberculosis activity. | Sharipova et al., 2018 |
| 14. | Xylose | Sugar | Synergistic antimicrobial effects with antibiotics against <i>Acinetobacter baumannii</i> and <i>Klebsiella pneumoniae</i> . | Hidalgo et al., 2018 |
| 15. | Erythritol | Sugar alcohol | Antibacterial activity against <i>Porphyromonas gingivalis</i> , <i>Actinomyces viscosus</i> , and <i>Aggregatibacter actinomycetemcomitans</i> | Zhang et al., 2019 |
| 16. | Palmitic Acid | Fatty acid | Antifungal activity against <i>S. apiospermum</i> | Jung et al., 2013 |
| 17. | Ethylene glycol | Alcohol | Antibacterial activity against <i>E. coli</i> | Moghayedi et al., 2017 |
| 18. | Propylene glycol | Alcohol | Antibacterial activity against <i>Streptococcus mutans</i> , <i>Enterococcus faecalis</i> , and <i>E. coli</i> | Nalawade et al., 2015 |
| 19. | Resorcinol | Alcohol | Antibacterial activity against <i>Staphylococcus aureus</i> | Joray et al., 2011 |
| 20. | Hydroquinone | Alcohol | Antibacterial activity against <i>Staphylococcus aureus</i> through cell wall and membrane damage | Ma et al., 2019 |
| 21. | Tyrosol | Alcohol | Antibacterial effects on <i>Pseudomonas aeruginosa</i> | Abdel-Rhman et al., 2015 |
| 22. | Pyrogallol | Alcohol | Antibacterial effects against <i>Pseudomonas putida</i> | Kocaçalışkan et al., 2006 |
| 23. | 1-Tridecanol | Alcohol | Antibacterial activity against <i>Staphylococcus aureus</i> | Togashi et al., 2007 |

Binding affinity of compounds with DdIA ranged from -4.45 to -11.7 Kcal/mol (**Supplementary Table 2**). Highest binding affinity (-11.7 Kcal/mol) was exhibited by quinic acid, 3-caffeoyl with lower inhibition constant (2.64 nM). Next, glucose-1-phosphate (-9.48) and quinic acid (-9.17 Kcal/mol) showed better binding affinity with 112.08 nM constant and 188.24 nM inhibition constant (Ki), respectively, with DdIA protein. Although, it is observed that D-cycloserine (reference compound) with DdIA has low binding affinity (-5.49) compared with the other three compounds that showed highest binding affinity. In case of MAPK1, quinic acid, 3-caffeoyl also exhibited highest binding affinity (-10.45 Kcal/mol) followed by sucrose (-8.46 Kcal/mol) and glucose-1-phosphate (-8.38 Kcal/mol). Ki of quinic acid, 3-caffeoyl (21.87 nM) with MAPK1 was slightly higher than with DdIA. In addition, binding affinity (-8.30 Kcal/mol) of Trametinib (reference compound) was

found less than other top three compounds with MAPK1. Further, structural interaction studies for bond formation were visualized in Discovery Studio. Quinic acid, 3-caffeoyl was able to form seven hydrogen bonds with Lys13, Gly47, His107 (two bonds), Leu110, Gly111, and Ser115 residues of DdIA proteins (**Figure 4A**). Glucose-1-phosphate and quinic acid have formed three and seven hydrogen bonds, respectively (**Figures 4B, C**). D-cycloserine formed only four hydrogen bonds, which include conventional and carbon hydrogen bonds (**Figure 4D**). Interaction of MAPK1 and quinic acid, 3-caffeoyl exhibited 10 hydrogen bonds with different amino acid residues, in which Glu105, Gly13, Cys166, and Asp 167 shared one bond each, whereas Lys51, Met107, and Asn154 formed two hydrogen bonds (**Figure 4E**). However, sucrose and glucose-1-phosphate were able to form five and seven hydrogen bonds, respectively, with MAPK1

TABLE 5 | Hydrogen bond and hydrophobic interaction of top six compounds with DdlA (*Xoo*) and MAPK1 (*M. oryzae*).

| Complex | BindingAffinity (Kcal/mol) | Hydrogen Bond | | | Hydrophobic | | |
|-------------------------------|-------------------------------|-----------------|------------------------|------------------|------------------|------------------------|--------------|
| | | BondingType | Protein | Distance (Å) | BondingType | Protein | Distance (Å) |
| | | | Interacting Amino acid | | | Interacting Amino acid | |
| Quinic acid, 3-caffeoyl | -11.7 | Conventional | LysA: 13 | 2.67 | Pi-Sigma | Leu:119 | 3.57 |
| | | | GlyA: 47 | 2.20 | | | |
| | | | HisA: 107 (2) | 2.37, 1.93 | | | |
| | | | LeuA: 110 | 1.81 | | | |
| | | | GlyA: 111 | 1.86 | | | |
| Glucose-1-phosphate-DdlA | -9.48 | Conventional | LysA: 13 | 2.02 | | | |
| | | | GlyA: 47 | 2.27 | | | |
| | | Carbon hydrogen | GlyA: 47 | 3.05 | | | |
| Quinic acid-DdlA | -9.17 | Conventional | LysA: 13 | 2.10 | | | |
| | | | GlyA: 47 | 2.24 | | | |
| | | | TrpA: 49 | 2.45 | | | |
| | | | HisA: 107 (2) | 1.92, 2.23 | | | |
| | | | LeuA: 110 | 1.75 | | | |
| Dcycloserin-DdlA | -5.49 | Conventional | GlyA: 111 | 1.79 | | | |
| | | | GluA: 16 | 1.73 | | | |
| | | | GluA: 112 | 1.76 | | | |
| | | Carbon hydrogen | AsnA: 317 | 1.77 | | | |
| | | GlnA:189 | 3.40 | | | | |
| Quinic acid, 3-caffeoyl-MAPK1 | -10.45 | Conventional | LysA: 52 (2) | 2.22, 5.81 | Pi-Sigma | Leu16 | 3.85 |
| | | | GluA: 105 | 2.02 | Pi-Alkyl | ValA: 37 | 4.99 |
| | | | MetA: 107 (2) | 2.0, 2.23 | | AlaA: 50 | 4.46 |
| | | | GlyA: 153 | 2.15 | | | |
| | | | AsnA: 154 (2) | 1.78, 1.87 | | | |
| | | | CysA: 166 | 3.02 | | | |
| Sucrose-MAPK1 | -8.46 | Conventional | AspA: 167 | 2.58 | | | |
| | | | GluA: 104 | 3.02 | | | |
| | | Carbon hydrogen | MetA: 107 (3) | 2.10, 2.25, 2.21 | | | |
| | | GluA: 104 | 2.63 | | | | |
| Glucose-1-phosphate-MAPK1 | -8.38 | Conventional | GluA:104 (3) | 1.98, 2.07, 2.23 | | | |
| | | | GluA: 105 | 1.81 | | | |
| | | | MetA: 107 (3) | 1.83, 2.0, 2.05 | | | |
| Trametinib-MAPK1 | -8.30 | Conventional | LysA: 52 | 2.22 | Pi-Sigma | ValA: 37 | 3.71 |
| | | | GluA: 104 | 1.99 | | LeuA: 156 | 3.76 |
| | | | Asp: 167 | 2.46 | Pi-Alkyl | LeuA: 29 | 5.34 |
| | | | | | | LysA: 52 | 5.24 |
| | | | | | | CysA: 166 | 5.22 |
| | | | | | Alkyl | LeuA:29 | 4.48 |
| | | | | | | AlaA: 50 | 5.26 |
| | | | | | | LeuA: 156 | 5.28 |
| | | | | | Amide-Pi Stacked | LeuA: 29 | 3.89 |

(Figures 4F, G). In the case of Trametinib and MAPK1 interaction, major bonds were alkyl and Pi-alkyl with only three hydrogen bonds (Figure 4H). Interestingly, Lys13 and Gly47 of DdlA have shared common bonding with quinic acid, 3-caffeoyl, glucose-1-phosphate, and quinic acid, whereas His107, leu110, and Gly111 formed bonds with both quinic acid, 3-caffeoyl and quinic acid. Similarly, Met107 residue of MAPK1 exhibited common bonding with all three compounds, viz., quinic acid, 3-caffeoyl, sucrose, and glucose-1-phosphate. Common sharing of amino acid with

different compounds suggests the key target amino acid residue of both the proteins. The high binding affinity of quinic acid, 3-caffeoyl for both DdlA and MAPK1 protein could be due to presence of caffeoyl group. Previous studies suggest that the increasing number of caffeoyl groups promoted anti-mutagenicity (Yoshimoto et al., 2002), which could be used as a target antimicrobial agent, because bacteria undergo several mutations during growth for survival. In addition, various studies confirmed that quinic acid, 3-caffeoyl inhibited the growth of fungus and

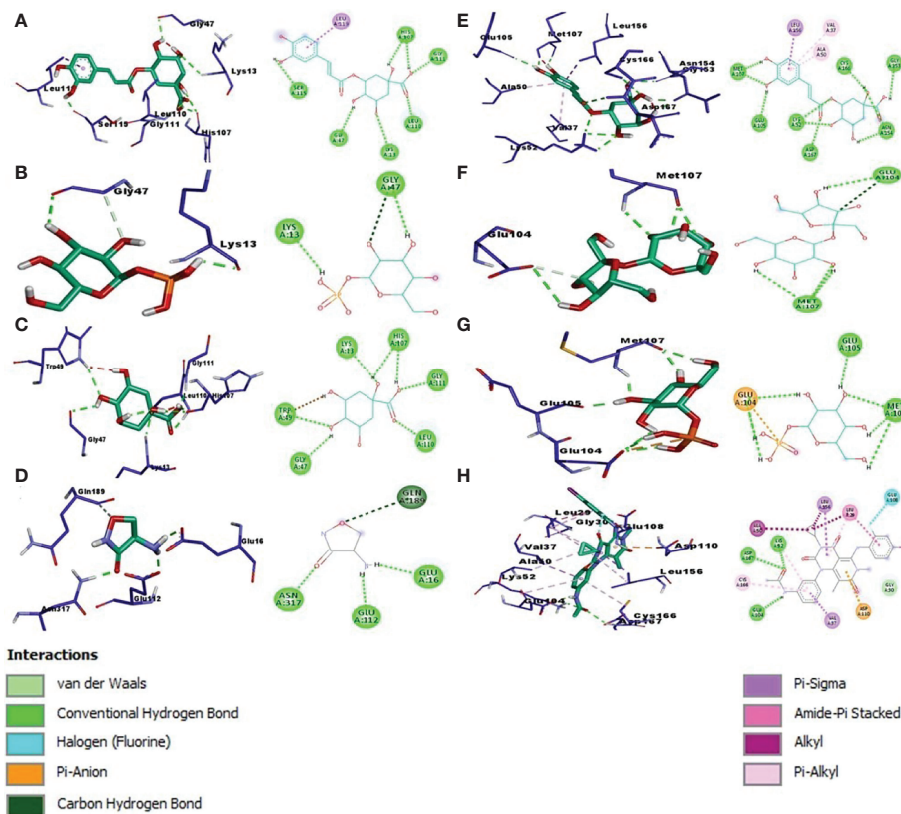


FIGURE 4 | Left panel of each alphabet showing 3D image while right panel indicating the 2D image. Molecular docking interaction and different bond formation of quinic acid, 3-caffeoyl (A), glucose-1-phosphate (B), quinic acid (C), and Dcycloserine (D) with DdlA protein of *Xoo*. Molecular docking interaction of quinic acid 3-caffeoyl (E), sucrose (F), glucose-1-phosphate (G), and Trametinib (H) with MAPK1 protein of *M. oryzae*.

bacteria (Karunanidhi et al., 2013; Martínez et al., 2017). So far, several phytochemical-based inhibitors have been predicted through molecular docking analysis against human and plant proteins responsible for disease (Tahir et al., 2019; Basu et al., 2020; Joshi et al., 2021). Recently, Loliolide, Eicosapentaenoic acid, Salicylic Acid Methyl Ester, and Phytol have been reported as antimicrobial agents against MAPK1, PDF, and SUH of *M. oryzae* and *Xoo* (Mansoori et al., 2020). Binding energy and K_i were also represented through heat map, which indicates that all ligands have more affinity with DdlA than MAPK1 (Figure 5). However, a linear correlation between binding energy of DdlA and MAPK1 with metabolites showed a positive correlation ($R^2 = 0.82$), which indicates the almost equal potential of ligands for inhibition of target proteins (Supplementary Figure 3). Thus, molecular docking result indicates the higher efficiency of quinic acid, 3-caffeoyl and quinic acid for inhibition of both DdlA and MAPK1 proteins with consideration of their binding affinity and inhibition constant.

Risk Assessment of Extract Through ADMET Analysis

The ADMET study revealed the physicochemical nature of compounds, including the rules of five (MW, iLOGP, HBAs,

and HBDS), as well as other parameters like molecular polar surface area (TPSA), rotatable bonds (ROTBs), aromatic heavy atoms numbers, and number of alerts for undesirable substructure (i.e., PAINS #alert AND Brenk #alert). Four compounds were subjected to ADMET analysis and values were compared with the default range for each parameter (Isyaku et al., 2020). It was found that MW, MR, RB, and the number of heavy atoms for each compound were considerable, whereas TPSA was only considerable, for quinic acid (Table 6). In addition, there is no alert for PAINS and Brenk for quinic acid and sucrose, whereas one and two alerts of Brenk for glucose-1-phosphate and quinic acid, 3-caffeoyl respectively. Although, all the compounds are soluble in water, which is necessary for any compounds to be a drug or drug-like. Among all the four compounds, only quinic acid has fitted under default ranges of parameters. Hence, it can be stated that quinic acid could be used as a natural antioxidant and may serve as a safe compound for treatment of BB disease without harming the surrounding flora and fauna.

CONCLUSION

The current investigation found that both extracts (ME and AE of *U. indica*) have better and similar antioxidant and free radical

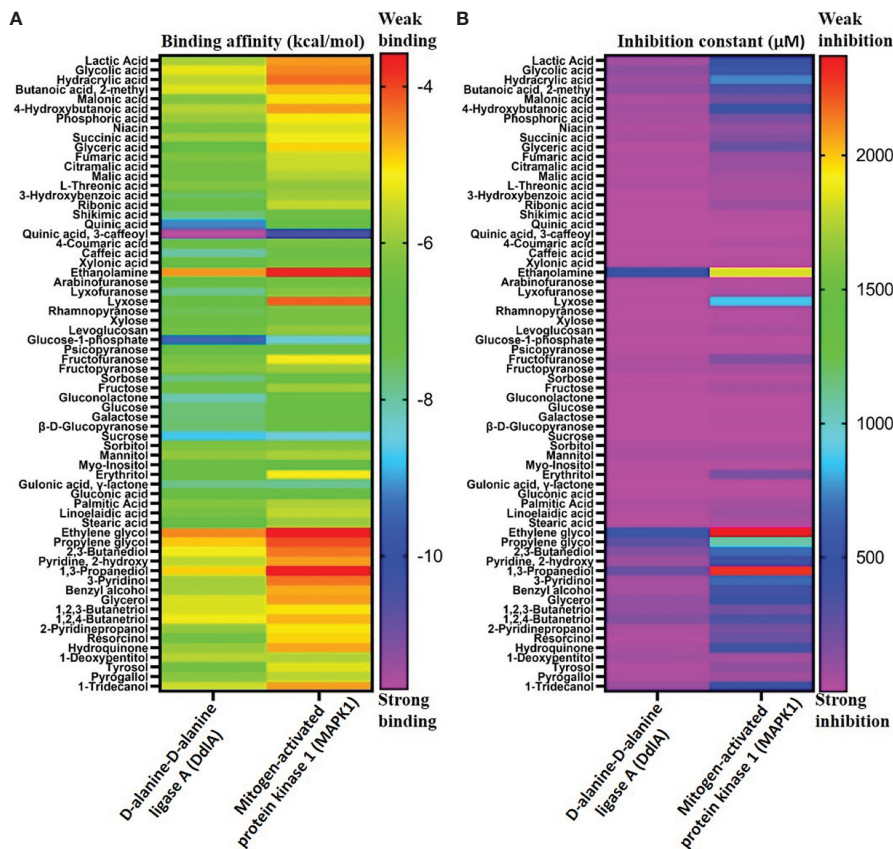


FIGURE 5 | Binding affinity of different metabolites with DdIA (*Xoo*) and MAPK1 (*M. oryzae*) protein (A). Inhibition constant of metabolites with DdIA (*Xoo*) and MAPK1 (*M. oryzae*) protein (B). Pink and red color representing strong and weak binding affinity, respectively. Heat map represents the strong binding affinity and inhibition constant of metabolites with DdIA (*Xoo*) than MAPK1 (*M. oryzae*).

scavenging properties. Both ME and AE contain useful phytochemicals such as alkaloids, flavonoids, quinones, coumarin and steroids, saponins, and terpenoids, in which total phenol, alkaloid, flavonoid, and antioxidant confirm its presence through quantitative analysis. The iron chelating activities of both the extracts were also found similar, and dose-dependent scavenging activity of NO and DPPH was also noted. The correlation study

indicates that NO radical scavenging activity was positively correlated with TAA and iron chelating activity. Similarly, DPPH radical scavenging activity was also found to be positively correlated with ABTS radical scavenging activity, TFC, and TPC. These results suggest that *U. indica* could be used to avert free radical-mediated disease occurrence, amelioration, and therapies. The FTIR spectrum analysis indicated the presence of several

TABLE 6 | Calculated ADMET parameters of the compounds.

| Parameter/property Name | Default Range | Compounds | | | |
|---|---------------|-------------------------|----------------------|--------------|--------------|
| | | Quinic acid, 3-caffeoyl | Glucose-1- Phosphate | Quinic Acid | Sucrose |
| Molecular weight | 50–500 | 354.31 g/mol | 260.14 g/mol | 192.17 g/mol | 342.30 g/mol |
| Octanol/water partition coefficient (iLOGP) | –2–10 | 0.96 | –0.44 | –0.12 | 1.04 |
| Topological polar surface area (TPSA) | 20–130 | 164.75 | 166.72 | 118.22 | 189.53 |
| Number of H- Bond acceptor (HBAs) | 0–10 | 9 | 9 | 6 | 11 |
| Numbers of H- Bond donors (HBDs) | 0–5 | 6 | 6 | 5 | 8 |
| Rotatable bonds (RB) | 0–5 | 5 | 3 | 1 | 5 |
| Number of heavy atoms | 15–50 | 6 | 16 | 13 | 23 |
| Molar refractivity (MR) | 40–130 | 83.50 | 46.65 | 40.11 | 68.16 |
| Water solubility | – | Soluble | soluble | Soluble | Soluble |
| PAINS (#alert) | – | 1 | 0 | 0 | 0 |
| Brenk (#alert) | – | 2 | 1 | 0 | 0 |

functional groups that are integral part of various secondary metabolites such as phenol and flavonoids, which could be responsible for the extract's antimicrobial and antioxidant properties of the extract. The GC-MS investigation for metabolite profiling revealed the presence of several compounds with antimicrobial activity against different human and plant pathogens. Further, molecular docking study showed promising binding affinity of phytochemicals like quinic acid, 3-caffeoyl, glucose-1-phosphate, quinic acid with DdIA protein, and quinic acid, 3-caffeoyl, sucrose, and glucose-1-phosphate with MAPK1 of *Xoo* and *M. oryzae*, which could be used to target pathogen receptors to control the rice pathogens. The highest binding affinity of quinic acid, 3-caffeoyl for both DdIA and MAPK1 protein could be due to presence of caffeoyl group. In addition, ADMET analysis revealed the quinic acid as their drug-like behavior. Hence, it can be stated that quinic acid could be used as a natural antioxidant and may serve as a safe compound for treatment of BB disease and rice blast without harming the surrounding flora and fauna. However, more research studies, such as the identification and isolation of reliable specific antimicrobial and antioxidant compounds, are needed and recommended for its broad-spectrum application as well to validate the specific activity against rice pathogens.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

AK, AM, AD, and SKD designed the article. AD, AM, KS, and SKD performed the work and wrote the article. AK and TT

corrected the article and finally, all authors read and approved the final article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2022.922306/full#supplementary-material>

Supplementary Figure 1 | | Calibration curve of Gallic acid (A), Quercetin (B) and Ascorbic acid (C).

Supplementary Figure 2 | | Pearson's correlation coefficient (r) test among TPC, TFC, TAA, DPPH, ABTS, nitric oxide radicals scavenging activity, and Iron chelating activity. Correlation coefficients are indicated by the color (Red: positive correlation; blue: negative correlation). TPC = total phenol content, TFC = total flavonoid content, TAA = total antioxidant activity, NO = nitric oxide inhibition, IC = iron chelation.

Supplementary Figure 3 | | Summary of linear correlation between binding affinity of DdIA and MAPK1 in terms of Kcal/mol.

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