



ORIGINAL ARTICLE

Polyphenolic characterization and evaluation of multimode antioxidant, cytotoxic, biocompatibility and antimicrobial potential of selected ethno-medicinal plant extracts



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KEYWORDS

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Abstract *Introduction:* Scientific evidence about biological profile of natural products can support their traditional uses. The current work was aimed to assess phytochemical and biological profile of nine medicinal plants collected from Herbalists.

Methods: Extracts prepared in different solvents were subjected to phytochemical, antioxidant, enzyme inhibitory, cytotoxic, and antimicrobial activities. Reverse phase-high performance liquid chromatography (RP-HPLC) analysis was performed for the quantification of polyphenols.

Results: Results showed methanol extract (M) being potent as compared to others. *Gentian lutea* M showed maximum extract recovery (15.00 ± 0.11 % w/w) and TFC (30.82 ± 0.21 µg QE/mg extract). *Nigella sativa* M displayed highest TPC (44.99 ± 0.43 µg GAE/mg extract) and TAC (334.72 ± 0.35 µg AAE/ mg extract). Results showed noteworthy quantities of vanillic acid, rutin, kaempferol, emodin in ethyl acetate (EA) and methanol (M) extracts of plants assessed by RP-HPLC. Gentisic acid was highest (11.75 µg/mg extract) in *T. arjuna* M extract. Similarly, maximum %FRSA (82.28 ± 0.03 %) and TRP (160.40 ± 0.38 µg AAE/ mg extract) were depicted by *Ter-*

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minalia chebula and *Chamomilla recutita*, respectively. Moreover, *Mentha longifolia* and *G. lutea* M demonstrated noteworthy ($p < 0.05$) antibacterial activity against *Staphylococcus aureus* (14 ± 0.7 mm) and *Klebsiella pneumoniae* (12 ± 0.3 mm), respectively. *Curcuma amada*, *C. recutita*, *Murraya koenigii* and *G. lutea* M had significant α -glucosidase activity. Another good solvent for extraction was ethyl acetate (EA), whose extracts were secondary to methanol in producing significant biological profile. For example, EA of *N. sativa* (TPC: 1.46 ± 0.45 μ g GAE/ mg extract), *G. lutea* (TRP: 160.33 ± 0.52 μ g AAE/mg extract; ZOI of 12 ± 0.5 mm in *K. pneumoniae*) and *Mormodica charantia* (α -amylase inhibition: 39.5 ± 0.10 %) showed significant bioactivities. All extracts displayed mild antifungal protein kinase inhibition activities and were significantly ($p < 0.05$) cytotoxic to brine shrimps with negligible hemolytic activity.

Conclusion: Briefly, variable polarity solvent extracts of studied plants will be processed for isolation of antioxidant, cytotoxic, carbohydrate enzyme inhibitory and antibacterial compounds.

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1. Introduction

The current world is plagued with plethora of diseases. These range from minor ailments like cough and flu to major diseases like life threatening infections and cancer. Although, multiple medicines are available to cater the needs of people; however, there are issues of efficacy, side effects and compliance. To furnish the medical needs, there is continuous research on new medicines either from synthetic or natural sources. The most replenishing source is the medicinal plants containing multitude of phytochemicals, which can be used as supplements, supportive remedies or as treatment of particular diseases (Wadood et al. 2013, Dias et al. 2012). Historically medicinal plants serve as the foundation of traditional herbal medicine. There are about 422,000 flowering plant species world-wide, out of which approximately 50,000 are considered as an integral part of traditional herbal medicine (Rashid et al. 2021). In developed countries, about 80 % of the world's people are totally dependent on herbal drugs for primary healthcare needs, and more than 25 % of the prescribed medications are derived from wild plant species (Schultes and Hofmann 1992). A recent study showed that consumption of cruciferous vegetable can prevent colorectal cancer formation and reduce its incidence (Ağagündüz et al. 2022). Moreover, medicinal plants including *Abelmoschus esculentus* (Haque et al. 2022), *Linum usitatissimum* and *Coriandrum sativum* (Mechchate et al. 2021) are reported for their antihyperglycemic activities. Researchers have also demonstrated the role of *Spirulina platensis* and *Ophiorrhiza rugose* in the management of pain (Chy et al. 2021, Freitas et al. 2021). All these studies indicate the clinical importance of medicinal plants and natural products obtained from those. Therapeutic applications of natural products have always spiked the interest of researchers that facilitated the discovery process of innovative and effective compounds (Bibi et al. 2017).

Natural products are basically the phytochemicals that have variable physical, chemical and biological properties (Khan et al. 2019, Goyal et al. 2011). Chemically, phytochemicals include mixture of primary and secondary metabolites such as proteins, carbohydrates, alkaloids, glycosides, terpenoid, phenolics and flavonoids (Wadood et al. 2013). These have different physical natures when extracted, i.e., as gums, semi-solids, crystals of alkaloids etc. Furthermore, these phytochemicals have reported significant biological properties including immunomodulatory, antioxidant, anti-inflammatory, enzyme stimulation/inhibition, antimicrobial, antiplatelet and hormonal regulating activities (Zohra et al. 2019). For instance, Küpeli Akkol et al., have reported the significance of coumarin polyphenols in cancer (Küpeli Akkol et al. 2020). polyphenols found in natural products exhibit free radical scavenging ability with inhibition of enzymes accountable for production of reactive oxygen species (ROS). Phenolic compounds have gained an attention as potent agents in controlling and treating oxidative stress related diseases including cardiovascular problems, inflammation, diabetes mellitus, cancer, and microbial diseases (Li et al. 2014). In vivo studies on polyphenols demonstrated that these exert their function by controlling plasma

membranes permeability/integrity, acting as transcription factors and regulating enzymatic activities (Selby-Pham et al. 2017). Despite the significance of phytochemicals, it is always mandatory to evaluate or repurpose the properties of phytochemicals since these are obtained from various medicinal plants or from the same plant collected during different seasons. Thus, a battery of up-to-date isolation and phytochemical evaluation methods are utilized to provide a validated scientific data on bioactive natural products (Omotayo and Borokini 2012, Goyal et al. 2011).

Some of the important natural products have been obtained from *Terminalia (T) arjuna*, *Momordica (M) charantia* and *Curcuma (C) amada*. The *M. charantia*, commonly called bitter melon, has been reported with antioxidant, anti-inflammatory, hepatoprotective, anti-hyperglycemic, antibacterial, immunomodulatory, antiulcer, and anti-tumor activities that can be attributed to the presences of various phytochemicals like flavonoids, phenols, and terpenoids (Naqvi et al. 2020). *T. arjuna* (*Combretaceae*) is used as aphrodisiac, diuretic, expectorant, cardioprotective, tonic and as supplement in cancer patients (Javed et al. 2016). *C. amada*, commonly known as Mango ginger, possess curcuminoids, amadaldehyde, phenolic acids and amadannulen exhibiting anti-inflammatory, brine-shrimp lethality, analgesic and antiplatelet activities (Policegoudra et al. 2011).

Following these lines, this work was designed to assess the phytochemical parameters and biological potential of *Mentha (M) longifolia*, *Gentiana (G) lutea*, *Nigella (N) sativa*, *T. arjuna*, *Chamomilla (C) recutita*, *M. charantia*, *Murraya (M) koenigii*, *C. amada* and *Terminalia (T) chebula*. We assessed the phytochemicals in different extracts prepared using solvents of variable polarities and evaluated the antimicrobial, cytotoxic, enzyme inhibition, blood biocompatibility and antioxidant activities of selected plants. This study is a good contribution to research in natural products and provides scientific evidence of some of the traditional uses of selected plants.

2. Methods

2.1. Plant collection and verification

Native plants *M. longifolia*, *G. lutea*, *N. sativa*, *T. arjuna*, *C. recutita*, *M. charantia*, *M. koenigii*, *C. amada* and *T. chebula* (Table 1) were collected from local herbalists of Islamabad, Pakistan. All plants were verified by Prof. Dr. Rizwana Aleem Qureshi, Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan. Specified herbarium number was assigned to all plants including *M. longifolia* (PHM-527), *G. lutea* (PHM-528), *N. sativa* (PHM-529), *T. arjuna* (PHM-530), *C. recutita* (PHM-531), *M. charantia* (PHM-532), *M. koenigii* (PHM-533), *C. amada*

Table 1 Botanical and ethnopharmacological profile of selected plants.

Botanical name	Local name	Part used	Family	Ethno medicinal uses	References
<i>Mentha longifolia</i>	Mint, podina	Aerial parts	Lamiaceae	Carminative, indigestion, wound healing, anti-microbial, anti-inflammatory, stomachache, antimutagenic, anti-arthritis, carminative and colitis.	(Farzaei et al., 2017)
<i>Gentian lutea</i>	Pakhan peid	Roots	Gentianaceae	Effective for stomach, anti-inflammatory, wound healing, gastritis and antioxidant.	(Niiho et al., 2006, Mathew et al., 2004)
<i>Nigella sativa</i>	Kalonji	Seeds	Ranunculaceae	Inflammation, gastro disorders, cancers, antioxidant properties, analgesic, anti-diarrheal, antimicrobial, cardiovascular disorders, anticarcinogenic and antidiabetic properties.	(Ahmad et al., 2013, Majeed et al., 2021)
<i>Terminalia arjuna</i>	Arjun	Bark	Combretaceae	Anti-ulcerative and anti <i>H.pylori</i> effect, antioxidant, anti-inflammatory, immunomodulatory, Anticarcinogenic, antimutagenic potential, antiatherogenic and cardiovascular activities.	(Javed et al. 2016)
<i>Chamomilla recutita</i>	Gul-e- baboon	Flower	Asteraceae	Active against <i>H. pylori</i> , analgesic, hepato protective, GIT disorders, anti-inflammatory, carminative, antispasmodic properties, wound-healing, and antimicrobial effects.	(Singh et al., 2011, Zadeh et al., 2014)
<i>Mormodica charantia</i>	Kareela	Fruit	Cucurbitaceae	Antimalarial, antidiabetic, pneumonia, psoriasis, cancer, infectious diseases, asthma, ulcers, bronchitis, anti-neoplastic properties, cholera, anemia and gout.	(Naqvi et al. 2020)
<i>Murraya koenigii</i>	Curry patta, Mitha Neem	Leaves	Rutaceae	Antidiabetic, anti-cancer, antioxidant, stomachic, carminative, antihelminthic, analgesic, antimicrobial activity, blood purifier, antidepressant, antidiarrheal	(Handral et al., 2012, Saini and Reddy, 2015)
<i>Curcuma amada</i>	Amba haldi, mango ginger	Rhizome	Zingiberaceae	Inhibition of <i>H.pylori</i> growth, antioxidant, antimicrobial, hypoglycemic and cyto-toxic activity	(Policegoudra et al. 2011)
<i>Terminalia chebula</i>	Hareer	Fruit	Combretaceae	Antimicrobial (<i>Anti H. pylori</i>), antidiabetic, antiretroviral, antioxidant, antiparasitic, antitumour, diuretics, antitussive and wound healer	(Das et al., 2020, Nigam et al., 2020)

(PHM-534) and *T. chebula* (PHM-535). Shade dried specimen of subject plants were archived within Herbarium of Medicinal Plants, Quaid-i-Azam University, Islamabad.

2.2. Chemicals and solvents

Ethyl acetate, Methanol and Distilled water (analytical grade) were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, Alpha amylase, Alpha glucosidase, and 2, 2-diphenyl, 1-picrylhydrazine (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals were procured from Merck (Darmstadt, Germany), unless the source is specified.

Ethical statement

The experiments where human blood was required, was performed after approval from Ethical Committee of Quaid-i-Azam University (No. BEC-FBS-QAU2021-259) and in consensus with the WHO guidelines for drawing blood (2010).

Informed consent was taken from all individuals who volunteered for blood sampling.

2.4. Crude extract preparation

Selected plants were rinsed, dried in shade (37 °C; 3 weeks), powdered with commercial miller and stored in a sealed container until use. Accurately weighed plant powder (750 g) was soaked in 1500 ml analytical grade solvent using Erlenmeyer flask at room temperature (20–25 °C) for 72 h and extracted by maceration with frequent sonication at the temperature 25 °C (frequency 25 kHz; two cycles; 30 min) for 24 h. The Marc was filtered, filtrates concentrated (rotary evaporator; Buchi, Switzerland), vacuum dried (Yamato, Japan) at 45 °C and stored at – 20 °C for use.

2.5. Estimation of extraction yield

The percent extract recovery of dried extracts was estimated using the following formula.

Extract recovery (%) = $X/Y \times 100$

Where, X is the total weight of dried extract and Y is total weight of plant powder used for extraction.

2.6. Phytochemical investigation

2.6.1. Folin–Ciocalteu method for total phenolic content estimation (TPC)

Folin–Ciocalteu reagent was used for estimating the total phenolic content following reported protocols (Bray et al. 1952). Concisely, individual extracts (20 μ l; 4 mg/ml DMSO) and Folin–Ciocalteu reagent (90 μ l) were incubated for 5 min in a 96 well microtiter plate and subsequently mixed with sodium carbonate (90 μ l; 6 % w/v). Absorbance values were measured at 630 nm with Elx 800 microplate reader (Biotech USA). Calibration curve was prepared using gallic acid (3.125–50 μ g/ml) as internal control ($y = 0.0282x + 0.0189$, $R^2 = 0.9972$) (Ul-Haq et al. 2012). The assay was done in triplicate, and outcomes are stated as μ g gallic acid equivalent per mg of dry weight extract (μ g GAE/mg extract).

2.6.2. Total flavonoid content (TFC) measurement via aluminium chloride

Colorimetric analysis by means of aluminum chloride was conducted to determine total flavonoid content according to previously described protocols (Phull et al. 2021). Briefly, crude extract (20 μ l; 4 mg/ml DMSO), potassium acetate (1.0 M; 10 μ l), aluminum chloride (10 %; 10 μ l) and distilled water (160 μ l) were combined for 30 min at 37 °C in 96 well plate. Using microplate reader (Elx 800; Biotech USA), the reading was taken at 415 nm and calibration curve ($y = 0.0329x + 0.0369$; $R^2 = 0.9954$; $p < 0.05$) was drawn using twofold dilutions of quercetin (2.5–40 μ g/ml). After testing the extracts in triplicate, the results of flavonoid content are reported in μ g quercetin equivalent per mg of dry weight extract (μ g QE/mg extract).

2.7. Reverse-phase high performance liquid chromatography (RP-HPLC) of polyphenols

Polyphenol constituents in the crude extracts were quantified by RP-HPLC as reported previously (Ullah et al. 2014a, Nasir et al. 2017) using Agilent Chem Station and Zorbex C8 analytical column (4.6 \times 250 nm and 5 μ m particle size) equipped with DAD detector (Agilent technologies, Germany). Mobile phases A & B contained acetonitrile: methanol: water: acetic acid in ratios of 5:10:85:1 and 40:60:0:1, respectively. Mobile phases were eluted through the column at the flow rate of 1 ml/min. The mobile phase was eluted at different gradients. The gradient of mobile phase changed over a period of 30 min where concentration of mobile phase A declined from 100 to 0 % while mobile phase B was increased from 0 to 100 %. The gradient was run as follows in terms of mobile phase B: Initially for 0–20 min at 0–50 % B, then for 20–25 min at 50–100 % B and lastly for 25–30 min at isocratic 100 % B. Extracts (20 μ l; 10 mg/ml) were filtered using 0.45- μ m membrane filters and injected into the column with 10 min reconditioning phase between two samples. Methanol was used to recondition the columns. Phenolic standards including emodin, vanillic acid, myricetin, syringic acid, rutin,

catechin, coumaric acid, kaempferol and gentisic acid were prepared in concentrations of 10, 20, 50, 100, 200 μ g/ml in methanol. The UV absorption spectra of samples was recorded at 368 nm (myricetin and kaempferol), 325 nm (gentisic acid), 279 nm (syringic acid, coumaric acid, emodin and catechin) and 257 nm (rutin and vanillic acid). Polyphenols were quantified as μ g/mg of sample from the calibration curve. The limits of detection and quantification were calculated by formula $3.3 \times (\sigma/b)$ and $10 \times (\sigma/b)$, respectively. Here, σ and b stand for the standard deviation of response and slope of the calibration curve, respectively.

2.8. Biological assessment

2.8.1. Free radical scavenging activity (%FRSA)

Scavenging action of the extracts was evaluated by measuring its ability to quench the stable DPPH radical (Fatima et al. 2015). The capacity of extracts (4 mg/ml DMSO; 7.406–200 μ g/ml) to discolor (37 °C for 30 min) purple colored DPPH (0.092 mg/ml methanol) was measured at 517 nm using microplate reader (Elx 800; Biotech USA) to quantify the percent free radical scavenging activity (%FRSA) by following formula.

$$\%FRSA = (1 - X_s/X_c) \times 100$$

Where, X_s is the sample absorbance and X_c is the absorbance of the negative control.

Ascorbic acid was utilized as a positive control and IC_{50} values were calculated for each extract by GraphPad Prism 9 software.

2.8.2. Ammonium molybdate reduction capacity of extracts

Total antioxidant capacity (TAC) of all extracts was evaluated by measuring the reducing capacity of ammonium molybdate. The absorbance of a mixture of extract or ascorbic acid (100 μ g/ml) or DMSO (0.1 ml; 4 mg/ml of DMSO) and reagent (900 μ l, H_2SO_4 0.6 M, sodium phosphate 28 mM, ammonium molybdate 4 mM) stabilized at 95 °C for 90 min. (Ul-Haq et al. 2012) was measured at 695 nm. The mixture was stabilized at 95 °C for 90 min. After cooling, absorbance was measured at 695 nm using Agilent Technologies 8354 spectrophotometer (Germany) was used for triplicate analysis and all the results were calculated as μ g of ascorbic acid equivalent per mg of dry weight extract (μ g AAE/mg extract) (Phull et al. 2021).

2.8.3. Determination of total reducing power by potassium ferricyanide colorimetric analysis (TRP)

TRP of extracts was estimated following reported method (Zahra et al. 2017). Briefly, the extract (200 μ l; 4 mg/ml DMSO; $n = 3$) was incubated for 20 min with phosphate buffer (400 μ l; 0.2 M; pH 6.6) and potassium ferricyanide (500 μ l; 1 % w/v) at 50 °C. After centrifuging (3000 rpm for 10 min) with 10 % trichloroacetic acid (400 μ l), the supernatant (500 μ l) was combined with o ferric chloride (100 μ l; 0.1 %) and distilled water (500 μ l). The absorbance of all samples was recorded at 700 nm. DMSO and ascorbic acid replaced extracts as negative and positive controls, respectively. TRP of the extracts was presented as mg of ascorbic acid equivalent per mg of dry weight extract (mg AAE/mg extract).

2.8.4. Inhibition of carbohydrate metabolizing enzymes

2.8.4.1. α -Amylase blocking activity. The extracts analyzed for α -amylase blocking activity followed previously reported procedure (Fatima et al. 2022b). In each well of 96 well plate, a mixture of enzyme α -amylase (0.14 U/ml; 25 μ l), extract (10 μ l; 4 mg/ml DMSO), phosphate buffer (15 μ l; pH 6.8) and freshly prepared starch solution (40 μ l; 2 mg/ml) was stabilized at 50 °C for 30 min. To stop the further reaction, 1 M HCl (20 μ l) and iodine reagent (5 mM) were added. Positive and negative control wells had acarbose and DMSO replacing the extract, respectively, while in the blank well both enzyme and extract were replaced with an equal volume of phosphate buffer. After incubation, the absorbance was read at 540 nm (Elx 800 microplate reader; Biotech USA) and the activity was stated as percent inhibition of α -amylase per mg of dry weight of extract.

Enzyme inhibitory potential was estimated by the following equation:

$$\% \text{inhibition} = [(X_s - X_n) / (X_b - X_n) \times 100]$$

Where, X_s is the absorbance of test extracts, X_b is absorbance of blank well and X_n is absorbance of negative control.

2.8.4.2. α -Glucosidase blocking activity. Procedure describe by Kim et al. (2000) was used to assess the α -glucosidase inhibition potential of the extracts (Kim et al. 2000). Substrate solution of p-nitrophenyl-D-glucopyranose (PNG; 25 μ l; 20 mM) was added in 96 well plate already filled with phosphate buffer (69 μ l; 50 mM). Afterwards, 1 μ l of enzyme (3 units/ml) and 5 μ l of extract (4 mg/ml DMSO) were mixed and an initial reading was obtained at 405 nm with microplate reader (Elx 800; Biotech USA). After incubating samples at 37 °C for 30 min, the reaction was stopped using 100 μ l of sodium bicarbonate (0.2 mM) and absorbance was again measured at 405 nm. Acarbose worked as a positive control and in blank both enzyme and extract were replaced with phosphate buffer. Percent of inhibition for α -glucosidase action was analyzed using the formula given below:

$$\% \text{inhibition} = [X_s - X_n / X_b - X_n \times 100]$$

X_s = sample absorbance, X_b = blank absorbance and X_n = negative control absorbance. The test was carried out in triplicate.

2.8.5. Cytotoxicity assays

2.8.5.1. Brine shrimp lethality assay. Brine shrimps (*Artemia salina*) analysis is an easy tool to appraise the cytotoxic effect of crude extracts (Ahmed et al. 2017). Briefly, eggs of *A. salina* (Ocean star, USA) were bred in artificial sea water (38 g/l plus yeast 6 mg/l) over a period of 2448 h in bi-compartment plastic plate ensuring required illumination, oxygen supply and a temperature of 30–32 °C. The hatched nauplii were collected by a Pasteur pipette in a beaker having sea salt water. In a 96 well plate, 10 nauplii along with 150 μ l of sea salt water were added. Sequential dilution (200, 100, 50 and 25 μ g/ml; n = 3) of extracts (DMSO < 1 %) were added in nauplii containing well and filled to maximum of 300 μ l with ocean water. Doxorubicin (4 mg/ml stock; final concentrations: 10, 5, 2.5 and 1.25 μ g/ml; n = 3) and DMSO (1 %) worked as positive and negative controls, respectively. Then, after incubating for 24 h at 37 °C, the extent of nauplii death was assessed by counting the number of surviving nauplii under an inverted

microscope. Median lethal concentration (LC₅₀; n = 3) of studied extracts was evaluated using GraphPad Prism 9 software.

2.8.5.2. Protein kinase inhibition assay. The protein kinase inhibition test was conducted in triplicate by discerning the effect of extracts on hyphae growth of *Streptomyces 85E* strain (Yao et al. 2011). Fresh *Streptomyces 85E* spores were dispersed on sterile petri plates containing ISP4 medium. Sterile filter paper discs (6 mm) were soaked with (5 μ l; 20 mg/ml of DMSO; 100 μ g/disc) individual extracts and positioned on the plates implanted with *Streptomyces 85E*. An aliquote of 5 μ l of DMSO and Surfactin (4 mg/ml of DMSO; 20 μ g/disc) permeated discs worked as negative and positive controls, respectively. Later, sample plates were keep warm at 30 °C for 3 days and the results were recorded as bald (BZ) and clear zones (CZ) of inhibition surrounding the test sample and control discs.

2.8.6. Biocompatibility evaluation

2.8.6.1. Hemolytic assay. Hemolytic assay assessed the biocompatibility of selected plants extract with physiological system by following the procedure described by Nasar et al (2019) (Qasim Nasar et al. 2019). An aliquot of 1 ml human blood was centrifuged (14,000 rpm) to isolate RBCs. After discarding plasma, 200 μ l of pellet was re-suspended in phosphate buffer saline (PBS; 9.8 ml), centrifuged (2000 rpm; 10 min) and washed thrice (PBS). Later, erythrocyte suspension (100 μ l) was mixed with test extracts (200 μ l; 200, 100, 50, 25 μ g/ml) in PBS and incubated for 1 h at 35 °C in 96 well plate followed by centrifugation (1000 rpm) for 10 min. Absorbance of the supernatant (200 μ l; 96 well plate) was recorded at 450 nm by microplate reader. Triton X-100 (200 μ l; 0.5 %) and PBS (200 μ l; 1X) were the internal controls. Following formula was applied to calculate percent hemolysis.

$$\% \text{hemolysis} = \text{Abs of the test samples} / \text{Abs of the positive control} \times 100$$

2.8.7. Antimicrobial screening

2.8.7.1. Antibacterial assay. Antibacterial activity of selected plant extracts was tested against non-resistant bacrail strains by agar disc diffusion method (Madiha et al. 2016). Gram positive bacteria including *Staphylococcus (S) aureus* ATCC-6538 and *Bacillus (B) subtilis* ATCC-6633 and gram negative bacteria including *Escherichia (E) coli* ATCC-25922, *Klebsiella (K) pneumoniae* ATCC-1705 and *Pseudomonas (P) aeruginosa* ATCC- 15,442 were used in this assay. Filter discs permeated with extract (sterile; 5 μ l; 20 mg/ml DMSO), Ciprofloxacin, Cefixime (positive control; 4 mg/ml DMSO; 5 μ l) or DMSO (negative control; 1 %; 5 μ l) were positioned on fresh bacterial lawn (seeding density 1×10^6 CFU/ml) on nutrient agar. After the incubation for 24 h at 37 °C, the average diameter of zone of inhibition (ZOI) around each sample disc was recorded. Extracts creating ZOI \geq 12 mm were taken as active and subjected to minimum inhibitory concentration (MIC) determination using twofold microbroth dilution method (Kaushik and Goyal 2008). Serial dilutions of extracts (12.5–100 μ g /ml) in Mueller Hinton broth (Thermo Fischer Scientific, US) were incubated with individual bacterial strains (5×10^4 CFU/ml) in 96 well plate. After overnight incubation (37 °C), the MIC

was calculated by determining the optical density (OD) at 600 nm. The experiment was executed thrice.

2.8.7.2. Antifungal assay. Antifungal effect of the extracts was assessed in triplicate by agar disc diffusion method (Nasir et al. 2017). Fungal spores of the selected strains of *Aspergillus (A) niger* (FCBP-0198), *Aspergillus (A) fumigatus* (FCBP-66), *Aspergillus (A) flavus* (FCBP-0064), *Fusarium (F) solani* (FCBP-0253) and *Mucor (M)* species (FCBP-0300) were dispersed in Tween-20 solution (0.02 %) and concentration was matched with McFarland 0.5 turbidity. After swabbing 100 µl of individual strains on Sabouraud Dextrose Agar plates, sterile filter paper discs (60 mm) soaked with extracts (5 µl; 20 mg/ml DMSO) were positioned on the petri plates. DMSO and Terbinafine permeated discs served as internal controls. Subsequently incubating for 24–48 h at 28 °C, ZOI around the discs was measured. MIC was determined for extracts with ZOI ≥ 10 mm in diameter using 3.12–50 µg/disc concentrations (Nasir et al. 2017).

2.9. Statistical analysis

Results are presented as mean ± SD of respective values in triplicate. The results are analyzed by one way ANOVA using PASW Statistics 18 and GraphPad Prism 9 at significance values of $p < 0.05$, $p < 0.01$, or $p < 0.001$.

3. Results

3.1. Extraction yield versus extraction solvents

It is imperative to analyze the extractive yields of medicinal plants to ensure the efficacy of extraction process and the sol-

vent system used (Celeghini et al. 2001). The results showed variable extraction yields with different solvents as well as with the same solvent system (Fig. 1). The maximum extraction yield was exhibited by methanol (M) extract of *G. lutea* (15.0 ± 0.42 % w/w). It was followed by aqueous (A) extracts of *C. recutita* and *M. charantia*, and ethyl acetate (EA) extract of *G. lutea* with percentage recovery of 9.68 ± 0.52 , 8.06 ± 0.30 and 7.266 ± 0.38 % w/w, respectively. Conversely, the *M. koenigii* EA extract was recovered in least amount with 1.94 ± 0.22 % w/w of extraction yield.

3.2. Phytochemical investigation

Extracts prepared in different solvent may yield variable types or amounts of phytochemical. In general, polyphenolic compounds are considered essential for the biological profile of any medicinal plant. Phenolic compounds are the major class of secondary metabolites that have reported to possess strong correlation with antioxidant and anti-inflammatory activities of medicinal plants (Sarawong et al. 2014). Hence, phenolic compounds in all plant extracts were estimated *in vitro* in triplicate and calculated from the calibration curve of the respective standards.

3.2.1. Folin–Ciocalteu method for TPC

TPC analysis of all extracts showed that the phenolic compounds ranged from 44.99 ± 0.43 to 17.63 ± 0.32 µg GAE/mg extract (Fig. 2). The highest TPC was shown by *N. sativa* M (extract with value of 44.99 ± 0.43 µg GAE/mg extract ($p < 0.05$)). Likewise, M extracts of *T. chebula*, *C. amada* and *G. lutea* demonstrated 42.20 ± 0.23 , 41.42 ± 0.12 and 40.68 ± 0.45 µg GAE/mg extract, respectively of TPC. The EA extracts of *N. sativa* and *M. longifolia* showed

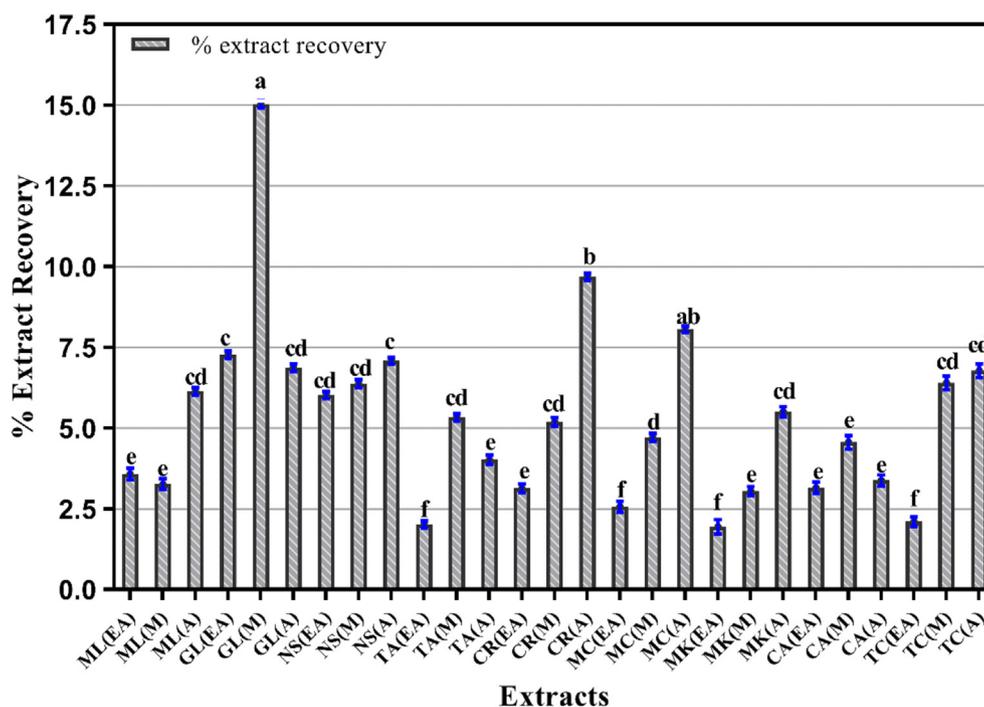


Fig. 1 Percent extract recovery of plant extracts using various solvent for extraction. Columns with different superscript (^{a-f}) letters show significantly ($P < 0.05$) different means. EA. Ethyl acetate; M. Methanol and A. Aqueous extracts.

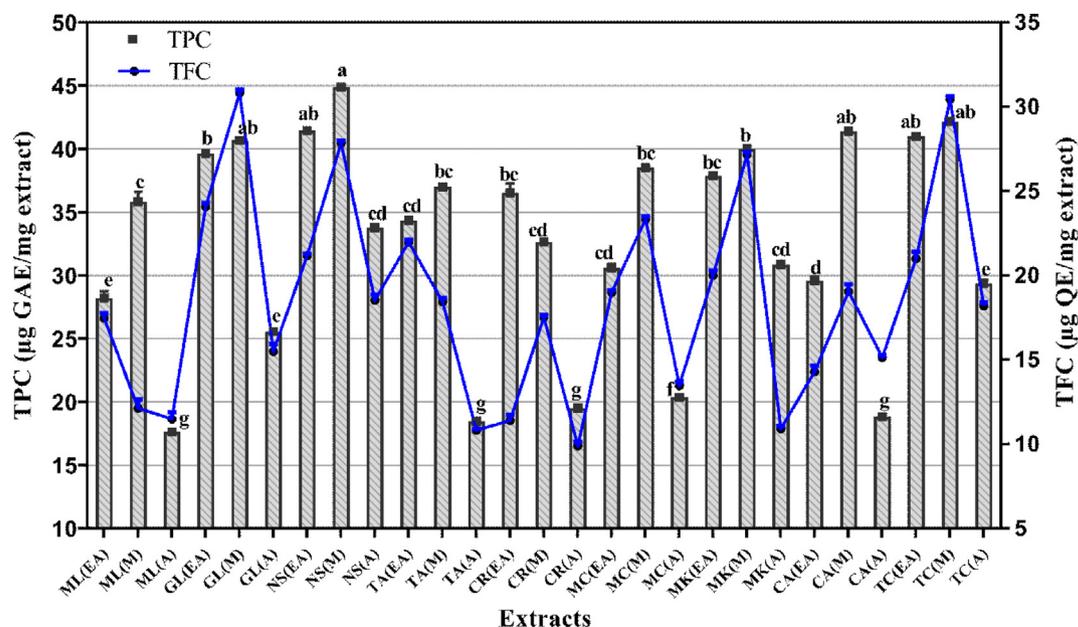


Fig. 2 Graphical presentation of total phenolic and total flavonoid content of all tested extracts. Values are accessible as mean \pm standard deviation from the triplicate analysis. Columns with different superscript (^{a-g}) letters show significantly ($P < 0.05$) different means. TPC. total phenolic content ($\mu\text{g GAE/mg extract}$); TFC. total flavonoid content ($\mu\text{g QE/mg extract}$); EA. Ethyl acetate; M. Methanol and A. Aqueous extracts.

41.46 ± 0.45 and $28.23 \pm 0.53 \mu\text{g GAE/mg extract}$ of TPC, respectively. The value gradually declined in A extracts of *N. sativa* ($33.80 \pm 0.12 \mu\text{g GAE/mg extract}$) and *T. chebula* ($29.37 \pm 0.17 \mu\text{g GAE/mg extract}$), whereas A extract of *M. longifolia* exhibited the least quantity of TPC ($17.63 \pm 0.32 \mu\text{g GAE/mg extract}$). Overall, the comparative decline in TPC of plant extracts was in the following order: M > EA > A (Fig. 2).

3.2.2. TFC measurement via aluminium chloride

Next, the assessment of TFC assay in all extracts demonstrated that M extracts of *G. lutea* ($30.82 \pm 0.21 \mu\text{g QE/mg extract}$) and *T. chebula* ($30.46 \pm 0.17 \mu\text{g QE/mg extract}$) showed the maximum value of TFC ($p < 0.05$). Among the EA extracts, *G. lutea* depicted significant TFC with value of $24.08 \pm 0.23 \mu\text{g QE/mg extract}$. On the contrary, the A extracts of *N. sativa* and *C. recutita* showed 18.54 ± 0.33 and $9.91 \pm 0.23 \mu\text{g QE/mg extract}$ of TFC, respectively where *C. recutita* had the least value. Overall, the A extracts showed less amount of TFC as compared with EA and M extracts.

3.3. Quantification of polyphenols

It is imperative to know about the concentrations of particular phytochemical in the extracts in order to relate the bioactivity profile. Moreover, the amount of phytochemical may directly relate with the extent of bioactivity and may represent the potency of that particular extract. Thus, polyphenols in test extracts were quantified by RP-HPLC procedure by comparing UV and retention time of 09 reference standards with extracts (Table 2). Results of HPLC-DAD analysis (Table 3) showed significant quantities of emodin, gentisic acid, kaempferol, myricetin and rutin within analyzed extracts. Maximum amount of emodin was expressed in A and M extracts of *G. lutea* (74.43 and $29.29 \mu\text{g/mg}$, respectively), whereas *T. arjuna* (M) exhibited maximum gentisic acid ($11.75 \mu\text{g/mg}$). A substantial quantity of kaempferol was also seen in EA and M extracts of *T. arjuna* with values of 8.39 and $4.94 \mu\text{g/mg}$, respectively. *M. koenigii* A and *T. arjuna* M extracts have 4.57 and $4.23 \mu\text{g/mg}$, respectively of myricetin. Chromatograms of detected phenols and standards are presented

Table 2 Polyphenolic compounds with retention time and wavelength.

Sr. No.	Polyphenols	Wavelength (nm)	Retention time	Limit of detection ($\mu\text{g/ml}$)	Limit of quantification ($\mu\text{g/ml}$)
1	Vanillic acid	257	9.714	0.0091	0.0277
2	Rutin	257	13.233	0.048	0.145
3	Catechin	279	7.463	0.066	0.200
4	Syringic acid	279	10.1	0.008	0.024
5	Coumaric acid	279	14.907	0.0025	0.0075
6	Emodin	279	29.125	0.080	0.242
7	Gentisic acid	325	8.233	0.0973	0.294
8	Myricetin	368	15.639	0.015	0.045
9	Kaempferol	368	21.387	0.038	0.115

Table 3 HPLC based quantification ($\mu\text{g}/\text{mg}$) of test plants extracts.

Plant name	Extract code	Vanillic acid	Rutin	Catechin	Syringic acid	Coumaric acid	Emodin	Gentisic acid	Myricetin	Kaempferol
<i>M. longifolia</i>	ML(EA)	1.89	Nd	0.23	0.38	0.10	Nd	Nd	0.31	2.66
	ML(M)	0.10	1.30	Nd	0.03	Nd	7.31	Nd	1.32	0.42
	ML(A)	0.10	0.40	Nd	Nd	Nd	Nd	Nd	Nd	1.28
<i>G. lutea</i>	GL(EA)	1.43	1.09	Nd	Nd	Nd	4.69	Nd	Nd	Nd
	GL(M)	Nd	1.54	Nd	Nd	Nd	29.29	Nd	Nd	Nd
	GL(A)	Nd	0.53	Nd	0.11	Nd	74.43	Nd	Nd	0.17
<i>N. sativa</i>	NS(EA)	Nd	1.82	0.32	0.46	0.01	Nd	Nd	Nd	2.55
	NS(M)	0.46	Nd	Nd	0.15	0.01	1.77	Nd	0.06	0.83
	NS(A)	0.45	Nd	Nd	0.13	Nd	Nd	Nd	Nd	Nd
<i>T. arjuna</i>	TA(EA)	0.24	1.66	Nd	0.02	0.23	0.83	Nd	Nd	8.39
	TA(M)	0.74	Nd	1.11	0.70	0.07	Nd	11.75	4.23	4.94
	TA(A)	0.10	1.30	Nd	0.03	Nd	7.31	Nd	1.32	0.42
<i>C. recutita</i>	CR(EA)	0.57	0.61	Nd	Nd	Nd	5.97	Nd	0.00	0.76
	CR(M)	Nd	2.57	Nd	0.04	Nd	12.96	Nd	Nd	Nd
	CR(A)	Nd	2.74	Nd	Nd	Nd	12.80	Nd	Nd	Nd
<i>M. charantia</i>	MC(EA)	0.90	0.24	2.24	0.04	0.02	Nd	3.52	0.63	Nd
	MC(M)	0.23	4.63	1.29	Nd	0.11	Nd	Nd	2.86	Nd
	MC(A)	0.87	2.30	0.30	0.07	0.01	Nd	4.89	Nd	Nd
<i>M. koenigii</i>	MK(EA)	Nd	1.46	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	MK(M)	0.45	Nd	Nd	0.13	Nd	Nd	Nd	Nd	Nd
	MK(A)	1.23	0.69	1.21	0.08	0.61	Nd	Nd	4.57	Nd
<i>C. amada</i>	CA(EA)	1.49	1.35	Nd	0.52	0.06	1.72	Nd	Nd	Nd
	CA(M)	Nd	2.69	0.87	0.07	Nd	Nd	Nd	0.34	Nd
	CA(A)	1.396	6.25	13.47	1.12	Nd	0.28	3.88	0.30	0.99
<i>T. chebula</i>	TC(EA)	0.771	Nd	0.23	Nd	Nd	Nd	Nd	0.31	2.66
	TC(M)	0.105	1.30	Nd	0.36	Nd	7.31	Nd	Nd	Nd
	TC(A)	0.469	Nd	Nd	0.15	Nd	1.77	Nd	0.06	0.83

Mentha longifolia (ML), *Gentian lutea* (GL), *Nigella sativa* (NS), *Terminalia arjuna* (TA), *Chamomilla recutita* (CR), *Momordica charantia* (MC), *Murraya koenigii* (MK), *Curcuma amada* (CA), *Terminalia chebula* (TC). Nd means not detected. M = methanol; EA = ethyl acetate; A = aqueous.

in Fig. 3 (a-f). This indicates that variable polarity solvents affect the extracted quantities of polyphenols from each plant.

3.4. Biological activities

Extracts prepared in different extraction solvents may present variable bioactivity profile owing to the presence of particular phytochemicals. Since, the extracts showed significant presence of polyphenols and flavonoids; therefore, their bioactivities were assessed to identify the best extract and extraction solvent for further isolation of active compounds.

3.4.1. FRSA evaluation

The capability of crude extracts to neutralize DPPH radical is representative of their antioxidant capacity (Fig. 4). The maximum %FRSA was exhibited by M extracts of *T. chebula* ($82.28 \pm 0.03\%$), *N. sativa* ($81.65 \pm 0.09\%$), *M. charantia* ($80.83 \pm 0.02\%$), *C. amada* ($78.65 \pm 0.23\%$), *M. koenigii* ($76.38 \pm 0.02\%$) and *C. recutita* ($75.02 \pm 0.05\%$) where *M. koenigii* M extract had the least IC₅₀ value ($20.87 \pm 0.12\ \mu\text{g}/\text{ml}$; $p < 0.05$). Among other extracts, *G. lutea* EA extract had maximum %FRSA ($66.39 \pm 0.11\%$), whereas, *M. charantia* A extract showed least %FRSA ($24.61 \pm 0.15\%$).

3.4.2. TAC determination by ammonium molybdate reduction capacity

Subsequent evaluation of antioxidant activity by TAC test demonstrated that *N. sativa* M extract has the highest TAC with a value of $334.72 \pm 0.35\ \mu\text{g AAE}/\text{mg extract}$ (Fig. 5). Significant ($p < 0.05$) antioxidant capacities were shown by *M. koenigii* M and *C. amada* M extracts with values of 307.17 ± 0.75 and $256.48 \pm 0.44\ \mu\text{g AAE}/\text{mg extract}$, respectively. The A extracts of *M. charantia* ($71.52 \pm 0.28\ \mu\text{g AAE}/\text{mg extract}$) and *C. recutita* ($73.81 \pm 0.77\ \mu\text{g AAE}/\text{mg extract}$) exhibited minimum TAC. Overall, the trend in TAC values declined in following way: M > EA > A.

3.4.3. Evaluation of TRP by potassium ferricyanide colorimetric analysis

Assessment of TRP as another method for antioxidant activity evaluation confirmed the results obtained by previous two assays. TRP analysis showed that maximum reducing power was exhibited by *C. recutita* M and *G. lutea* EA extracts with the values of 160.40 ± 0.38 and $160.33 \pm 0.52\ \mu\text{g AAE}/\text{mg extract}$, respectively ($p < 0.05$; Fig. 5). Similarly, *T. arjuna* A extract ($159.94 \pm 0.53\ \mu\text{g AAE}/\text{mg extract}$) and *G. lutea* M extract ($159.36 \pm 0.12\ \mu\text{g AAE}/\text{mg extract}$) demonstrated significant antioxidant activity. Least TRP value of 42.36 ± 0 .

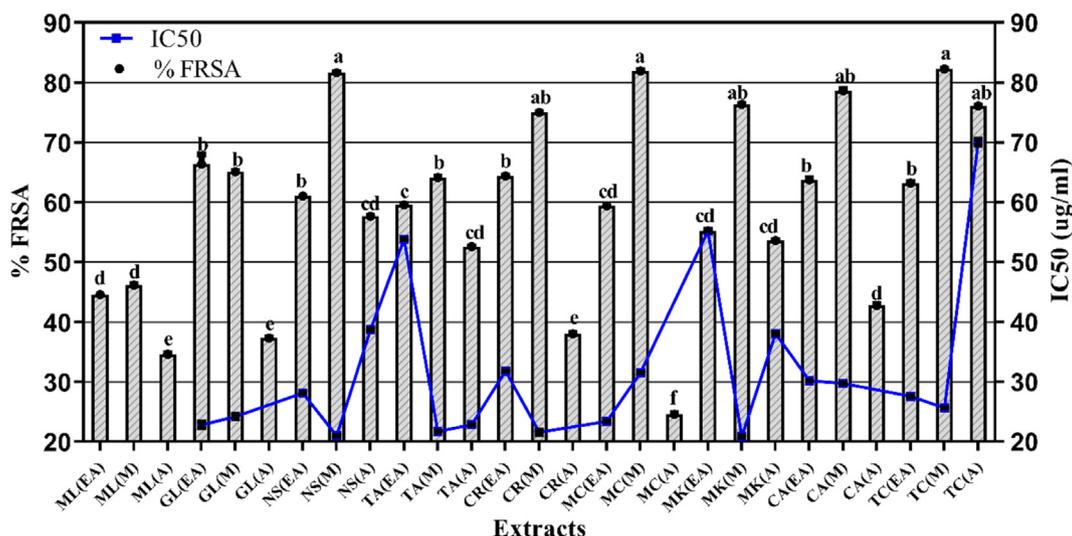


Fig. 4 Graphical presentation of free radical scavenging activity of tested extracts. Values are presented as mean \pm standard deviation from triplicate investigation. The columns with superscript (^{a-f}) letters displayed significantly ($P < 0.05$) different means. %FRSA. percent free radical scavenging activity; EA. Ethyl acetate; M. Methanol and A. Aqueous extracts.

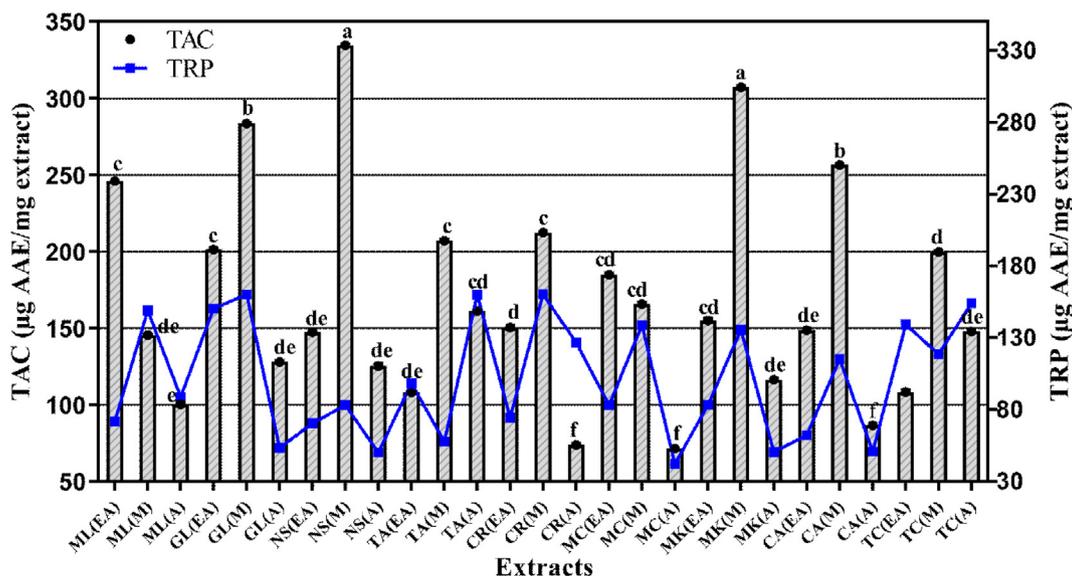


Fig. 5 Total antioxidant capacity and total reducing power of the selected plant extract. Values are presented as mean \pm Standard deviation ($n = 3$) and columns with different superscript (^{a-f}) letters show significantly ($P < 0.05$) different means. TAC. total antioxidant capacity; TRP. total reducing power; EA. Ethyl acetate; M. Methanol and A. Aqueous extracts.

29 $\mu\text{g AAE/mg extract}$ was expressed by A extract of *M. charantia*.

3.4.4. Inhibition of carbohydrate metabolizing enzymes

Carbohydrate metabolizing enzymes like α -amylase and α -glucosidase break complex carbohydrates to simple sugars and aid in the intestinal absorption of dietary carbohydrates. Studies have shown that antioxidant polyphenols in plant extracts can inhibit carbohydrate metabolizing enzymes and have beneficial effects in reducing blood glucose levels (Jemaa et al. 2017, Kalita et al. 2018). Thus, we next assessed the inhibitory effect of our extracts on α -amylase and α -

glucosidase enzymes to open a prospect for their potential antidiabetic profile.

3.4.4.1. α -Amylase blocking activity. Enzyme inhibition activity of all extract and acarbose showed significant variance from negative control ($p < 0.05$; Fig. 6). Acarbose, the positive control, inhibited α -amylase enzyme activity by $59.14 \pm 1.7\%$. Comparatively, most significant ($p < 0.05$) inhibition was displayed by EA and M extracts of *M. charantia* ($39.5 \pm 0.10\%$ and $29.6 \pm 0.3\%$). Rest of the sample showed moderate or negligible activity. The EA extract of *N. sativa* and *C. amada* exhibited the percent inhibition of $18.4 \pm 0.1\%$ and $27.3 \pm$

0.5 %, respectively. Likewise, M extracts of *M. koenigii* and *C. recutita* showed mild to moderate α -amylase inhibition activity (25.4 ± 0.06 % and 24.9 ± 0.3 %, respectively). On the contrary, EA extracts of *M. longifolia* and *G. lutea* indicated least percent inhibition in this assay.

3.4.4.2. α -Glucosidase blocking activity. Next, another test was executed to calculate the α -glucosidase enzyme blocking potential of selected extract. Among all the samples, significant ($p < 0.05$) α -glucosidase inhibition was shown by M extracts of *C. amada*, *C. recutita*, *M. koenigii* and *G. lutea* with values of 47.0 ± 0.22 %, 47.5 ± 0.1 %, 46.1 ± 0.15 % and 42.3 ± 0.76 % inhibition, respectively (Fig. 6). Maximum inhibition was exhibited by A extract of *T. arjuna* (48.3 ± 0.12 % inhibition). A substantial activity was exhibited by EA extracts of *T. chebula* (31.4 ± 0.12 %) and *G. lutea* (38.0 ± 0.22 %). On the other hand, *T. arjuna* EA and *C. amada* A extracts showed least inhibition of α -glucosidase enzyme (Fig. 6).

3.4.5. Cytotoxicity assays

Assessment of *in vitro* toxicity of extract provides information about their safety as well as possibility of use as targeted therapy in cancer. Since, the extracts exhibited significant antioxidant activity; hence, their capacity to impede oxidative stress may aid in combating various diseases. Consequently, we used brine shrimp and protein kinase inhibition assays to depict the likelihood of extracts for further investigation in cancer models.

3.4.5.1. Brine shrimp lethality assay. Cytotoxic potential assessment in brine shrimps showed significant ($p < 0.05$) results for all extracts where most of the extract had greater than 80 % cytotoxicity at 200 μ g/ml (Table 4). *M. longifolia* A extract had least LC₅₀ value of 48.15 ± 0.13 μ g/ml. This indicates that it is most potent among all other extracts. Furthermore, *C. recutita* EA, *G. lutea* A, and *M. koenigii* EA extracts also dis-

played potent cytotoxic action with LC₅₀ values of 50.58 ± 0.12 , 48.47 ± 0.15 and 49.00 ± 0.19 μ g/ml, respectively. Doxorubicin (6.25 ± 1.25 μ g/ml) was still most potent among all tested samples while the negative control DMSO showed no toxicity. Overall, the extracts LC₅₀ values ranging between 48 and 96 μ g/ml.

3.4.5.2. Protein kinase inhibition assay. Another test to estimate the cytotoxic potential was inhibition of protein kinases that are responsible for hyphae formation in *Streptomyces 85E* strain. Results (Table 4) showed moderate to low protein kinase inhibition with maximum bald zone of 13 ± 0.38 mm shown by *M. koenigii* EA extract. It was followed by *M. charantia* EA (11 ± 0.10 mm) and *M. koenigii* M (11 ± 0.13 mm) extracts. Rest of the extracts had mild protein kinase inhibition activity with bald zones between 7 and 9 mm. It appears that non-polar solvent extracts have some phytochemicals that are more effective in inhibiting hyphae formation than polar solvent extracts.

3.4.6. Biocompatibility evaluation

3.4.6.1. Hemolytic assay. Hemolytic assay was executed to check the toxic effects of extracts on erythrocytes to ensure their safety profile. It was observed that there was 10–8 %, 8–6 %, 4–2 % and 3–1.5 % hemolysis at 200, 100, 50 and 25 μ g/ml concentrations (Table 4). The maximum hemolysis at 200 μ g/ml was shown by *T. arjuna* A extract with the value of 10.69 ± 0.82 % as compared to Triton-X 100 (positive control; 100 ± 0.63). There was dose dependent decline in the hemolytic effects of the extracts. This indicates that all the extracts are safe at ≥ 200 μ g/ml where majority of the biological activities are observed.

3.4.7. Antimicrobial activities

Prevention and management of infections standalone or associated with other diseases like diabetes, cancer and injuries

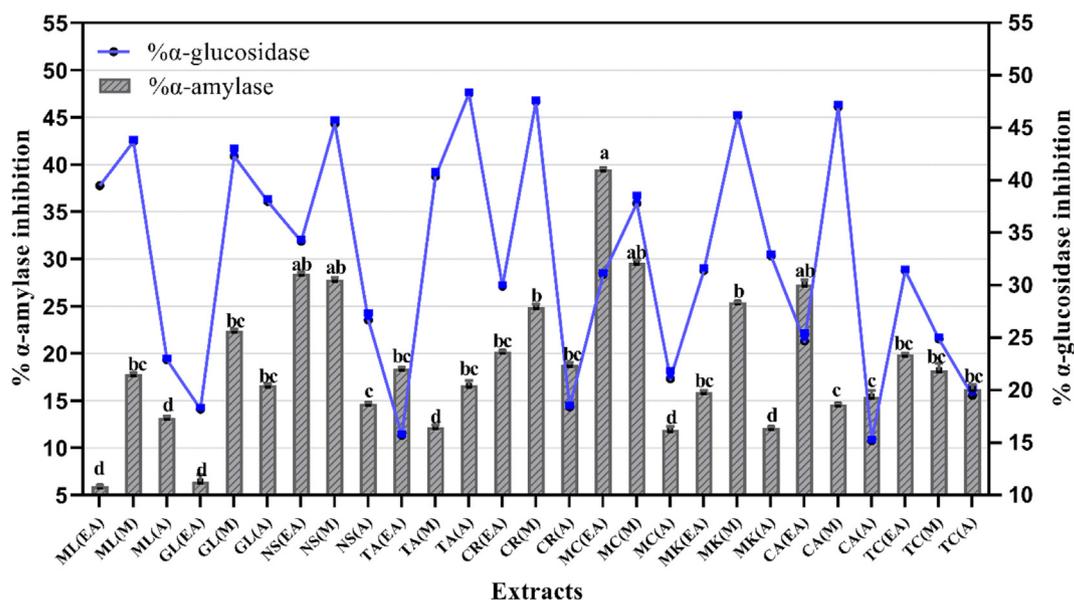


Fig. 6 Inhibition of carbohydrate metabolizing enzymes by selected plant extracts. Values are presented as mean \pm Standard deviation ($n = 3$). The columns with different superscript (^{a-d}) letters show significantly ($P < 0.05$) different means. EA, Ethyl acetate; M, Methanol and A, Aqueous extracts.

Table 4 Cytotoxic and hemolytic potential of selected plant extracts.

Plant name	Extract code	Brine shrimp cytotoxicity		Protein kinase inhibition		%Hemolytic analysis			%Hemolysis	
		% Mortality	LC-50	Diameter (mm) 100 µg/disc	Bald zone	Clear zone	(200 µg/ml) %Hemolysis	(100 µg/ml) %Hemolysis	(50 µg/ml) %Hemolysis	(25 µg/ml) %Hemolysis
<i>M. longifolia</i>	ML(EA)	90 ± 5.77 ^a	70.71 ± 0.21	8 mm ± 0.30 ^{cd}			9.13 ± 0.58 ^{ab}	7.43 ± 0.34 ^{cd}	3.86 ± 1.42 ^c	2.74 ± 0.21 ^f
	ML(M)	90 ± 0.11 ^a	59.62 ± 0.19	–		7 mm ± 0.53 ^d	9.56 ± 0.54 ^b	6.55 ± 0.30	4.35 ± 0.70 ^{de}	2.31 ± 0.15 ^{fg}
	ML(A)	80 ± 0.51 ^b	48.15 ± 0.13	–		8 mm ± 0.21 ^{cd}	10.42 ± 0.36 ^a	8.49 ± 0.36 ^{cd}	4.24 ± 0.82 ^{de}	3.17 ± 0.17 ^{ef}
<i>G. lutea</i>	GL(EA)	90 ± 5.72 ^a	51.00 ± 0.61	7 mm ± 0.4 ^d		–	9.72 ± 0.94 ^b	8.11 ± 0.34 ^{cd}	3.97 ± 0.89 ^c	2.74 ± 0.06 ^f
	GL(M)	70 ± 1.02 ^c	56.19 ± 0.89	8 mm ± 0.18 ^{cd}		–	9.08 ± 0.82 ^{ab}	7.79 ± 0.33 ^d	4.56 ± 1.12 ^{de}	3.17 ± 0.15 ^{ef}
	GL(A)	90 ± 0.00 ^a	48.47 ± 0.19	7 mm ± 0.22 ^d		–	9.40 ± 0.56 ^{ab}	8.33 ± 0.36 ^{cd}	4.29 ± 0.39 ^{de}	3.38 ± 0.03 ^{ef}
<i>N. sativa</i>	NS(EA)	90 ± 0.69 ^a	50.00 ± 0.74	–		7 mm ± 0.11 ^d	8.38 ± 0.74 ^{cd}	7.52 ± 0.52 ^d	3.97 ± 0.82 ^c	2.79 ± 0.09 ^f
	NS(M)	80 ± 0.58 ^b	48.24 ± 0.26	8 mm ± 0.17 ^{cd}		–	8.91 ± 0.68 ^c	6.34 ± 0.36 ^{de}	2.20 ± 0.61 ^{fg}	1.77 ± 0.05 ^g
	NS(A)	80 ± 0.00 ^b	59.62 ± 0.39	–		8 mm ± 0.42 ^{cd}	9.56 ± 0.82 ^b	8.17 ± 0.34 ^{cd}	3.54 ± 0.32 ^c	2.36 ± 0.12 ^{fg}
<i>T. arjuna</i>	TA(EA)	90 ± 0.10 ^a	83.86 ± 0.19	7 mm ± 0.19 ^d		–	9.88 ± 1.22 ^b	7.52 ± 0.56 ^d	4.08 ± 0.59 ^{de}	3.06 ± 0.03 ^{ef}
	TA(M)	60 ± 1.77 ^d	88.99 ± 0.97	9 mm ± 0.30 ^c		–	9.61 ± 0.94 ^b	6.82 ± 0.39 ^{de}	3.92 ± 0.41 ^c	2.84 ± 0.18 ^f
	TA(A)	90 ± 0.00 ^a	49.02 ± 0.32	–		8 mm ± 0.28 ^{cd}	10.69 ± 0.82 ^a	7.47 ± 0.46 ^{cd}	2.68 ± 0.77 ^f	2.04 ± 0.16 ^{fg}
<i>C. recutita</i>	CR(EA)	90 ± 0.13 ^a	50.58 ± 0.12	9 mm ± 0.61 ^c		–	10.58 ± 0.82 ^a	8.76 ± 0.36 ^c	4.29 ± 0.82 ^{de}	2.31 ± 0.15 ^{fg}
	CR(M)	80 ± 0.00 ^b	49.02 ± 0.31	–		8 mm ± 0.9 ^{cd}	10.80 ± 0.94 ^a	7.74 ± 0.36 ^d	3.86 ± 0.58 ^c	3.33 ± 0.09 ^{ef}
	CR(A)	90 ± 0.31 ^a	70.71 ± 0.15	10 mm ± 0.77 ^{ab}		7 mm ± 0.36 ^d	10.37 ± 0.86 ^a	8.60 ± 0.31 ^c	3.54 ± 0.82 ^c	2.90 ± 0.07 ^f
<i>M. charantia</i>	MC(EA)	70 ± 5.77 ^c	50.00 ± 0.23	11 mm ± 0.10 ^{ab}		7 mm ± 0.14 ^d	10.26 ± 0.82 ^a	8.49 ± 0.16 ^{cd}	4.51 ± 1.82 ^{de}	3.33 ± 0.10 ^{ef}
	MC(M)	90 ± 0.27 ^a	50.00 ± 0.82	–		7 mm ± 0.7 ^d	9.77 ± 0.57 ^b	8.01 ± 0.36 ^{cd}	4.24 ± 0.82 ^{de}	3.65 ± 0.9 ^e
	MC(A)	60 ± 1.75 ^d	88.99 ± 0.19	–		7 mm ± 0.24 ^d	10.42 ± 1.42 ^a	6.66 ± 0.36 ^{de}	2.68 ± 0.76 ^f	2.20 ± 0.05 ^{fg}
<i>M. koenigii</i>	MK(EA)	80 ± 0.64 ^b	49.00 ± 0.19	13 mm ± 0.38 ^a		–	9.99 ± 0.82 ^b	7.25 ± 0.32 ^{cd}	4.83 ± 0.43 ^{de}	2.68 ± 0.06 ^f
	MK(M)	90 ± 0.00 ^a	59.62 ± 0.27	11 mm ± 0.13 ^{ab}		–	9.67 ± 0.69 ^b	8.92 ± 0.36 ^c	3.38 ± 0.85 ^{ef}	1.82 ± 0.14 ^g
	MK(A)	90 ± 0.23 ^a	70.57 ± 0.91	9 mm ± 0.52 ^c		7 mm ± 0.19 ^d	10.20 ± 0.80 ^a	7.04 ± 0.38 ^{cd}	3.81 ± 0.62 ^c	2.74 ± 0.7 ^f
<i>C. amada</i>	CA(EA)	90 ± 0.00 ^a	70.71 ± 0.26	–		12 mm ± 0.10 ^{ab}	9.08 ± 0.72 ^b	8.87 ± 0.36 ^c	4.19 ± 0.49 ^{de}	3.27 ± 0.04 ^{ef}
	CA(M)	90 ± 0.44 ^a	96.94 ± 0.15	7 mm ± 0.15 ^d		10 mm ± 0.41 ^b	9.45 ± 1.15 ^{ab}	7.47 ± 0.32 ^{cd}	3.92 ± 0.82 ^c	2.95 ± 0.09 ^f
	CA(A)	60 ± 1.73	96.71 ± 0.89	–		7 mm ± 0.73 ^c	10.53 ± 1.05 ^a	8.65 ± 0.31 ^c	4.35 ± 0.88 ^{de}	2.63 ± 0.5 ^f
<i>T. chebula</i>	TC(EA)	90 ± 0.77 ^a	48.24 ± 0.53	8 mm ± 0.22 ^{cd}		–	8.97 ± 1.17 ^c	8.11 ± 0.36 ^{cd}	3.81 ± 0.56 ^c	2.84 ± 0.6 ^f
	TC(M)	90 ± 5.61 ^a	49.02 ± 0.91	7 mm ± 0.36 ^d		–	8.59 ± 1.01 ^c	6.45 ± 0.37 ^{de}	3.27 ± 0.42 ^{de}	1.55 ± 0.9 ^g
	TC(A)	80 ± 0.58 ^b	56.19 ± 0.34	8 mm ± 0.11 ^{cd}		–	9.94 ± 1.13 ^b	7.95 ± 0.31 ^d	4.62 ± 0.61 ^{de}	2.09 ± 0.11 ^{fg}
Surfactin										
Doxorubicin		96 ± 0.21 ^a	6.25 ± 1.25							
Triton-X							100 ± 0.63 ^a			
DMSO							0.5 %			

ZOI (Zone of inhibition) represented as diameter (mm) of clear zone and bald zone. Values are presented as mean from triplicate investigation ± standard deviation by triplicate investigation. The columns with different superscript (^{a-g}) letters show significantly (P < 0.05) different means, –: shows no activity. EA: Ethyl acetate, M: Methanol and A: aqueous extract.

require the use of multiple antibiotics. Plant extracts, which are safe and have good antioxidant and antimicrobial profile, can be alternative to allopathic medicines (Manso et al. 2021). Hence, afterwards we investigated the extracts for their potential antibacterial and antifungal activities.

3.4.7.1. Antibacterial assay. The antibacterial assay of plant extracts in different non-resistant bacterial strains showed mild to moderate activity (Table 5). The extracts with zone of inhibition (ZOI) ≥ 8 mm and ≤ 14 mm is considered moderately active, extracts with ZOI ≥ 15 mm and ≤ 21 is considered active, while the highly active extracts exhibited ZOI ≥ 22 mm and ≤ 29 mm. *M. longifolia* EA and M extracts exhibited significant ($p < 0.05$) activity against *S. aureus* with ZOI of 12 ± 0.4 mm and 14 ± 0.7 mm at $100 \mu\text{g}/\text{disc}$. EA and

M extracts of *G. lutea* also revealed significant ($p < 0.05$) effect against *K. pneumoniae* with ZOI of 12 ± 0.5 mm and 12 ± 0.3 mm, respectively. Likewise, A and M extracts of *T. chebula* presented a potent antibacterial activity against *K. pneumoniae* (13 ± 0.1 mm) and *P. aeruginosa* (14 ± 0.2 mm), respectively. This means that though moderate, the extracts showed variable antibacterial profile against different strains that possibly will be accredited to the existence of diverse phytochemicals. No ZOI was present in negative control that established that DMSO did not affect the results whereas Ciprofloxacin (14 ± 0.10 mm to 18 ± 0.24 mm) and Cefixime (23 ± 0.1 m m to 26 ± 0.6 mm) showed highly significant ($p < 0.05$) antibacterial activities. The MIC value of $50 \mu\text{g}/\text{disc}$ was observed for M extract of *M. charantia* and M and EA extracts of *M. longifolia* against *S. aureus*. Results indicated that M

Table 5 Antibacterial activity of selected plant extracts.

Diameter (mm) of growth inhibition zone at $100 \mu\text{g}/\text{disc}$ and MIC ($\mu\text{g}/\text{ml}$)										
Extract code	<i>E. coli</i>	MIC	<i>K. pneumoniae</i>	MIC	<i>B. Subtilis</i>	MIC	<i>P. aeruginosa</i>	MIC	<i>S. aureus</i>	MIC
<i>M. longifolia</i>										
ML(EA)	–	–	–	–	–	–	$9 \pm 0.5^{\text{de}}$	–	$12 \pm 0.4^{\text{d}}$	50
ML(M)	–	–	$11 \pm 0.3^{\text{de}}$	–	–	–	–	–	$14 \pm 0.7^{\text{c}}$	50
ML(A)	–	–	$10 \pm 0.8^{\text{de}}$	–	–	–	–	–	–	–
<i>G. lutea</i>										
GL(EA)	–	–	$12 \pm 0.5^{\text{d}}$	50	–	–	–	–	–	–
GL(M)	–	–	$12 \pm 0.3^{\text{d}}$	100	$9 \pm 0.5^{\text{de}}$	–	–	–	$10 \pm 0.1^{\text{de}}$	–
GL(A)	–	–	$9 \pm 0.5^{\text{de}}$	–	–	–	–	–	$9 \pm 0.6^{\text{de}}$	–
<i>N. sativa</i>										
NS(EA)	$9 \pm 0.4^{\text{de}}$	–	–	–	–	–	–	$7 \pm 0.8^{\text{c}}$	–	–
NS(M)	–	–	–	–	$8 \pm 0.4^{\text{de}}$	–	$10 \pm 0.5^{\text{de}}$	–	$12 \pm 0.3^{\text{d}}$	–
NS(A)	–	–	–	–	$9 \pm 0.1^{\text{de}}$	–	–	–	–	–
<i>T. arjuna</i>										
TA(EA)	$8 \pm 0.5^{\text{de}}$	–	$10 \pm 0.3^{\text{de}}$	–	–	–	–	–	$10 \pm 0.5^{\text{de}}$	–
TA(M)	–	–	$12 \pm 0.6^{\text{d}}$	100	–	–	–	–	–	–
TA(A)	–	–	$9 \pm 0.8^{\text{de}}$	–	–	–	$11 \pm 0.5^{\text{d}}$	–	–	–
<i>C. recutita</i>										
CR(EA)	$8 \pm 0.5^{\text{de}}$	–	–	–	$10 \pm 0.3^{\text{de}}$	–	–	–	–	–
CR(M)	–	–	–	–	$8 \pm 0.5^{\text{de}}$	–	–	–	–	–
CR(A)	$7 \pm 0.5^{\text{e}}$	–	–	–	–	–	$9 \pm 0.5^{\text{de}}$	–	–	–
<i>M. charantia</i>										
MC(EA)	$8 \pm 0.5^{\text{de}}$	–	–	–	–	–	$11 \pm 0.2^{\text{de}}$	–	–	–
MC(M)	–	–	$8 \pm 0.5^{\text{de}}$	–	$10 \pm 0.4^{\text{de}}$	–	$11 \pm 0.3^{\text{de}}$	–	$14 \pm 0.2^{\text{c}}$	50
MC(A)	–	–	–	–	$9 \pm 0.5^{\text{de}}$	–	$11 \pm 0.5^{\text{de}}$	–	–	–
<i>M. koenigii</i>										
MK(EA)	–	–	$11 \pm 0.5^{\text{de}}$	–	$10 \pm 0.5^{\text{de}}$	–	$12 \pm 0.5^{\text{d}}$	25	$10 \pm 0.5^{\text{de}}$	–
MK(M)	$8 \pm 0.5^{\text{de}}$	–	$9 \pm 0.5^{\text{de}}$	–	$9 \pm 0.5^{\text{de}}$	–	–	–	–	–
MK(A)	$9 \pm 0.5^{\text{de}}$	–	$10 \pm 0.3^{\text{de}}$	$9 \pm 0.5^{\text{de}}$	–	–	$9 \pm 0.7^{\text{de}}$	–	–	–
<i>C. amada</i>										
CA(EA)	$8 \pm 0.5^{\text{de}}$	–	$10 \pm 0.5^{\text{de}}$	–	–	–	$7 \pm 0.5^{\text{e}}$	–	$9 \pm 0.6^{\text{de}}$	–
CA(M)	–	–	$9 \pm 0.5^{\text{de}}$	–	–	–	$11 \pm 0.1^{\text{de}}$	–	–	–
CA(A)	$10 \pm 0.5^{\text{de}}$	–	–	–	$9 \pm 0.4^{\text{de}}$	–	$9 \pm 0.6^{\text{e}}$	–	–	–
<i>T. chebula</i>										
TC(EA)	–	–	$10 \pm 0.5^{\text{de}}$	–	–	–	–	–	$7 \pm 0.5^{\text{e}}$	–
TC(M)	$9 \pm 0.5^{\text{de}}$	–	–	–	–	$7 \pm 0.5^{\text{e}}$	$14 \pm 0.2^{\text{c}}$	50	$9 \pm 0.3^{\text{e}}$	–
TC(A)	–	–	$13 \pm 0.1^{\text{cd}}$	50	$11 \pm 0.5^{\text{de}}$	–	–	–	–	–
Controls										
Ciprofloxacin	$14 \pm 0.4^{\text{b}}$	–	$16 \pm 0.6^{\text{b}}$	1.25	$16 \pm 0.6^{\text{b}}$	1.25	$18 \pm 0.9^{\text{b}}$	1.25	$14 \pm 0.6^{\text{b}}$	1.25
Cefixime	$26 \pm 0.6^{\text{a}}$	–	$23 \pm 0.2^{\text{a}}$	1.25	$26 \pm 0.2^{\text{a}}$	2.50	$24 \pm 0.7^{\text{a}}$	2.50	$23 \pm 0.2^{\text{a}}$	1.25
DMSO	–	–	–	–	–	–	–	–	–	–

Results are mean \pm standard deviation of bacterial growth inhibition zone that was measured including 6 mm of disc. Sample concentration on disc was $100 \mu\text{g}/\text{disc}$. n = 3; Columns with superscript (^{a-c}) letters displayed significant P value < 0.05 , different means, – means no activity.

extracts of most plants presented better antibacterial potential than others.

3.4.7.2. Antifungal assay. Subsequently, plant extracts were tested against five fungal strains and the results showed mild antifungal activity. ZOI for *A. niger* ranges between 7.00 ± 0.34 mm to 8.23 ± 0.55 mm. In case of the A extracts, *T. chebula* (9.00 ± 0.45 mm), *G. lutea* (9.00 ± 0.50 mm) and *C. recutita* (7.00 ± 0.42 mm) exhibited the slightly significant (Table 6; $p < 0.05$) activity against *Mucor species*. Moreover, A extracts of *T. arjuna* (9.00 ± 0.30 mm) and *C. amada* (9.00 ± 0.65 mm) displayed noteworthy antifungal potential against *F. solani*. Likewise, M extracts of *N. sativa*, *C. amada* and *T. chebula* showed mild ($p < 0.05$) activity against *A. niger* with ZOI of 8.00 ± 0.16 mm, 8.00 ± 0.58 mm, and 8.23 ± 0.55 mm,

respectively. Against *A. fumigatus*, EA extract of *T. chebula* (9.00 ± 0.31 mm) and *M. koenigii* (8.00 ± 0.13 mm) were slightly significant ($p < 0.05$). Terbinafine, positive control showed highly significant antifungal activity (ZOI: 15–18 mm; $p < 0.05$) against all test strains, whereas DMSO did not produce any effect.

4. Discussion

Treatment of diseases using herbal remedies has been in practice since ages. According to WHO the characteristics of safety, efficacy, cost effectiveness and easy accessibility have made natural products a major source in resolving primary health problems world-wide (Hussain et al. 2011). Pakistan has plenty of herbal resources where more than 1000 species

Table 6 Antifungal appraisal of selected plant extracts.

Diameter (mm) of growth inhibition zone (100 µg/disc)					
Extract code	<i>F. solani</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>Mucor</i> sp.
<i>M. longifolia</i>					
ML(EA)	7.00 ± 0.33^c	–	–	–	9.00 ± 0.21^{ab}
ML(M)	–	–	7.00 ± 0.41^{cd}	–	–
ML(A)	–	–	–	8.00 ± 0.35^b	–
<i>G. lutea</i>					
GL(EA)	–	–	8.00 ± 0.43^{cd}	–	–
GL(M)	7.00 ± 0.11^d	–	–	–	–
GL(A)	–	–	7.00 ± 0.45^c	–	9.00 ± 0.50^{ab}
<i>N. sativa</i>					
NS(EA)	8.00 ± 0.35^b	–	–	–	8.00 ± 0.22^d
NS(M)	–	8.0 ± 0.16^b	–	–	–
NS(A)	–	7.00 ± 0.65^{cd}	–	–	7.00 ± 0.31^c
<i>T. arjuna</i>					
TA(EA)	7.00 ± 0.51^d	–	–	7.00 ± 0.30^{cd}	8.00 ± 0.27^b
TA(M)	–	–	–	–	–
TA(A)	9.00 ± 0.30^{ab}	–	–	–	–
<i>C. recutita</i>					
CR(EA)	–	7.00 ± 0.43^c	–	–	–
CR(M)	–	–	–	–	–
CR(A)	–	–	–	–	7.00 ± 0.42^c
<i>M. charantia</i>					
MC(EA)	–	7.00 ± 0.36^c	–	7.00 ± 0.51^c	–
MC(M)	–	–	8.00 ± 0.21^d	–	–
MC(A)	7.00 ± 0.44^c	–	–	–	–
<i>M. koenigii</i>					
MK(EA)	–	–	8.00 ± 0.31^b	–	–
MK(M)	–	7.00 ± 0.34^{cd}	–	–	–
MK(A)	–	–	–	7.00 ± 0.47^c	–
<i>C. amada</i>					
CA(EA)	–	–	–	–	8.00 ± 0.51^{cd}
CA(M)	–	8.00 ± 0.58^b	–	–	–
CA(A)	9.00 ± 0.65^{ab}	–	–	–	8.00 ± 0.52^b
<i>T. chebula</i>					
TC(EA)	7.00 ± 0.41^c	–	9.00 ± 0.13^{ab}	–	–
TC(M)	–	8.23 ± 0.55^b	–	–	–
TC(A)	7.00 ± 0.53^{cd}	–	–	–	9.00 ± 0.45^{ab}
Controls					
Terbinafine	15.0 ± 0.1	14 ± 0.5	18 ± 0.14^a	17 ± 0.2^a	17.7 ± 0.1^a
DMSO	–	–	–	–	–

Zone of growth inhibition was measured with 6 mm of disc. The sample concentration was 100 µg/disc. Values are presented as mean \pm SD that was calculated in triplicate analysis. Superscript (^{a-d}) letters on columns means significantly ($p < 0.05$) different from negative control, –: shows no activity. *A. flavus* = *Aspergillus flavus*, *A. fumigatus* = *Aspergillus fumigatus*, *A. niger* = *Aspergillus. Niger* and *F. solani* = *Fusarium solani*.

with documented medicinal value are used by herbalists to cure different diseases. Natural products obtained from biologically active plants are either used as crude drugs or as isolated phytochemicals from the subject plant. It is very challenging to ensure the quality of natural products owing to the complex nature of phytochemicals (Chothani and Patel 2012). Researchers and pharmaceutical industries has made substantial contribution to investigate the biologically active phytochemicals and standardize the natural products with approved efficacy and safety (Khan and Rauf 2014). Since, the characteristics of natural products vary depending upon extracts solvents and composition of extracts ultimately affecting their biological profile (Ullah et al. 2014b); therefore, it is imperative to evaluate the biological profile of natural products to validate their efficacy and provide scientific evidence of their use in traditional remedies by herbalists (Sasidharan et al. 2011). Following these lines, we attempted to investigate the phytochemical and biological profile of nine medicinal plants collected from Herbalists in Islamabad, Pakistan. Here, we report medicinal value of selected crude extracts prepared in solvents of varying polarity. We performed a range of assays to prepare an initial profile for all plants that will direct towards bioactivity mediated isolation of efficacious compounds.

4.1. Extraction yield versus extraction solvents

A total of 27 crude extracts of nine plants were prepared by sonication assisted maceration technique. It is the simple, reproducible and an exhaustive process where phytochemicals can be extracted using solvents of varying polarity (Wojdyło et al. 2021). Although, different conventional techniques are employed for extraction process because of their efficiency, wide-range application and ease of use (Stalikas 2007); however, risk–benefit ratio of each technique has to be considered to achieve the better quality of extract. For instance, exposure to high temperatures during extraction can destroy bioactive glycosides and polyphenols (Mahmoudi et al. 2013). In the current study, sonication assisted maceration provided optimal conditions for penetration of solvent in particles of dry plant powder and extracting significant quantities of phytochemicals (Wojdyło et al. 2021). Using distilled water, methanol and ethyl acetate ensured the extraction of polar and non-polar phytochemicals in different extracts. It was observed that extraction efficiency of methanol was higher as compared to non-polar solvent, which was also reflected in overall biological profile of the extracts. However, greater extract yield may not always direct towards absolute biological action (Fatima et al. 2015) and more pronounced activity may be observed in lower yield extracts depending on the nature of extracted phytochemicals.

4.2. Phytochemical investigation

As the first step in our investigation, polyphenols were quantified in all extracts. Recognition of phytochemicals within selected plants can be correlated with respective biological activities. Flavonoids and phenols are widely accepted as therapeutic agents due to their antioxidant, and immunoprotective properties that can protect the body from environmental and endogenous toxins. Distinct classes of flavonoids

are isolated with significant biological interests such as antidiabetic, anticancer, antimicrobial, antimalarial, neuroprotective and anti-inflammatory. Involvement of flavonoids in routine diet is well recommended to reduce the risk of numerous life-threatening diseases for instance heart attack, diabetes mellitus and cancer (Ullah et al. 2020).

The current study revealed significant polyphenols in all extracts, with the highest content in methanol extracts of *G. lutea*, *T. chebula*, *N. sativa* and *M. koenigii*. Literature shows that phenolic acids can alter gene expression of certain proteins to induce cytotoxic, anti-mutagenic and anticancer effects (Ribarova and Atanassova 2005). Flavonoids can block the free radical generation pathway by inhibiting lipid peroxidation and also possess well established anti-inflammatory activity (Inomata et al. 2013). Most of the effects are attributed to the aromatic ring and hydroxyl groups in polyphenolic compounds (Trembl and Šmejkal 2016). Previous studies have reported a significant correlation between polyphenols and *in vivo* antitumor effect by regulating PI3K/AKT and MAPK/ERK pathways (Vuong et al. 2016).

4.3. RP-HPLC quantification

Preliminary screening of phytoconstituents give the clue for natural aptitude of different plant extracts. Through extraction process, various secondary products are obtained, responsible for therapeutic capacities of traditional plants in various diseases (Gurjar et al. 2012). The presence of polyphenols in EA, M and A extracts confirmed by RP-HPLC demonstrated significant quantities of coumaric acid, gentisic acid, kaempferol, emodin, catechin, rutin and synergic acid. These polyphenols have established pharmacological profile. For example, rutin, emodin, myricetin and coumaric acid exhibit antioxidant, anti-inflammatory and antibacterial activities (Dai and Mumper 2010). Gentisic acid has effective biological activities such as neuroprotective, anti-inflammatory, antimicrobial, and antioxidant interests that make it a good remedy for prospective treatment of different ailments (Choubey et al. 2015). Epidemiological findings also revealed a substantial relationship between the consumption of phenolic acids enriched diet and the prevention of diseases (Akilandeswari and Ruckmani 2016). Furthermore, Kaempferol, a dietary flavonoid, acts as potent chemo-preventive substance for lung, breast and gastric cancer by restricting multiple signaling pathways, boosting the apoptosis mechanism via the suppression of cell cycle genes and activating p53 function (Khan et al. 2021). Consequently, in recent study, differentiated fingerprinting using 9 polyphenolic standards was performed for selected plant extracts confirmed substantial quantities of polyphenols that may be responsible for the biological activities of selected plants.

4.4. Biological activities

4.4.1. Antioxidant activity

Oxidative stress is the root of majority of diseases. Oxidative stress was found related to cardiovascular diseases, diabetes, cancer, alzheimer's disease, kidney disorders and chronic obstructive pulmonary disease (Liguori et al. 2018). Plant extracts containing polyphenols have been known to combat oxidative stress by scavenging or blocking the free radical for-

mation (Dahlia et al. 2017, Saha and Verma 2016, Mammad et al. 2017). In line with this, antioxidant activity of the extracts was determined by measuring the DPPH free radical scavenging activity (FRSA), ammonium molybdate reduction capacity (TAC) and potassium ferricyanide colorimetric analysis (TRP). Previous studies have shown antioxidant (Sheng et al. 2018) anti-inflammatory (Bordoni et al. 2019) and antimicrobial activities (Ali et al. 2021) of the selected plants. Here, we re-evaluated antioxidant activity to compare the effects of different extraction solvents.

DPPH free radical scavenging capacity is detected by change in the color of DPPH from violet to yellow (Yazdanparast et al. 2008). This happens because it can accept electrons from electron donors such as phenolics and flavonoids (Ahmed et al. 2017). Out of 27 extracts, M and EA extracts exhibited well defined DPPH scavenging activity, while A extracts showed the least potential. This was significantly ($p < 0.05$) correlated with phenolic ($R^2 = 0.656$) and flavonoid ($R^2 = 0.447$) content estimated in this study. Literature also supports the current findings (Dahlia et al. 2017, Saha and Verma 2016, Mammad et al. 2017) of free radical scavenging by phenolic and flavonoid content of the plant extracts.

Subsequent analysis of TAC also provided evidence of better results in methanol extracts as compared to other solvent extracts. Maximal TAC values were in the range of 145.45 ± 0.76 to 334.41 ± 0.35 $\mu\text{g QE/mg extract}$. TAC assay measures the electron and hydrogen donating capacity of test samples (Kasote 2013). Electron or hydrogen donating molecules like carotenoids, alpha-tocopherol, cysteine, ascorbic acid, terpenoids, polyphenols and aromatic amines (with OH aromatic rings) possess the potential to conduct a series of redox reactions and are considered as good antioxidants. Correlation ($R^2 = 0.4431$) was found between TFC and TAC values and it can be concluded that the antioxidant capacity of extracts can be attributed to the significant presence of polyphenols and other phytochemicals in the M and EA extracts. Polyphenols have been previously reported to combat oxidative stress by reducing ROS activity (Stagos 2019). The mechanism of polyphenols involves the formation of peroxy radical by the exchange of hydrogen atom from polyphenol structure with oxygen or electron of the free radical, thus quenching their oxidation potential. Phenols have ability to obstruct oxidation of LDL that is associated with reduction in critical diseases e.g., leukemia, Alzheimer's disease, breast, gastrointestinal, and ovarian cancers (Mileo et al. 2016).

The antioxidant activity was further proved by measuring the reduction process of ferric (Fe^{+3}) to ferrous (Fe^{+2}) under the action of extracts that transformed the yellow color of test solution to green (Wang et al. 2016). All extracts possess proton donating phytochemicals that provided reducing power to the samples. It has been reported that flavonoids (naringenin and catechin), volatile oils (carvacrol, thymol and menthol), phenolic acid (caffeic acid and gallic acid), phenolics diterpenes (carnosic acid, carnosol and rosmanol) show good reducing power (Brewer 2011). Furthermore, it has been documented that natural products with high phenolic and flavonoid content show good reducing potential with beneficial effects on health (Akbari et al. 2022). Since, polyphenols are natural antioxidant compounds; these can protect cells from ROS induced damage by quenching mechanism. Consequently, these help to inhibit the process of lipid oxidation and protect human

body cells against the oxidative damage that leads to decreased risk of some oxidative-stress associated diseases, such as cardiovascular, cancer and neurodegenerative dysfunctions (Afshari and Sayyed-Alangi 2017). A recent study showed that rutin in combination with vitamin C reduced $\text{TNF}\alpha$ levels, inflammation and oxidative stress in hemodialysis patients (Omar et al. 2022). Experimental evidence supports that coumaric acid inhibits melanin synthesis in murine melanoma cells and human epidermal cells and has implications in reducing pigmentation in human study (Boo 2019). Presence of these polyphenols in our extracts indicate that the selected extracts have can be likely used to manage inflammation and pigmentation and become the source for isolation of antioxidant, anti-inflammatory and antipigmentation compounds. Concisely, it can be concluded from the results that polar (methanol) plant extracts have notable antioxidant proficiency as compared to the other solvent extracts.

4.4.2. Catalytic inhibition of carbohydrate metabolizing enzymes

Carbohydrates are the primary source of energy for metabolic reactions in the body. Carbohydrates are metabolized by α -amylase and α -glucosidase enzymes to simple sugars. α -Amylase hydrolyzes α bonds of α -linked polysaccharides like starch and glycogen and converts those into glucose and maltose. α -glucosidase primarily catalyzes the hydrolysis of dietary starch into glucose for intestinal absorption. These provide sufficient glucose in blood that can cause post-prandial hyperglycemia in patients of diabetes mellitus (Zinjarde et al. 2011) and can be considered promoters of diabetes complications (Sangeetha and Vedaasree 2012). Hence, herbal remedies inhibiting α -amylase and α -glucosidase enzymes are effective strategy to minimize exaggerated spikes of post-prandial hyperglycemia. The present study showed that methanol and aqueous extracts have inhibited α -amylase and α -glucosidase enzyme activity *in vitro*. Methanol extracts of *N. sativa*, *C. amada*, *C. recutita*, and *M. koenigii* and aqueous extract of *T. arjuna* have shown significant enzyme inhibition. These results are supported by reports of antidiabetic activity of quercetin, rutin, and vanillic acid in *N. sativa* (Dalli et al. 2021), myricetin and kaempferol in *M. koenigii* (Ashokkumar et al. 2013) and luteolin and quercetin in *C. recutita* (Kato et al. 2008). Thus, in our study, potential anti-hyperglycemic activity of the polar solvent extracts can be linked to the diverse polyphenols in sample. Literature also demonstrates that effects linked with diabetes could be attributed to existence of phenolic content and also relate with antioxidant capability of commonly used herbs and medicinal plants (Ranilla et al. 2010). Furthermore, *C. amada*, *N. Sativa* and *M. charantia* have been traditionally used for the treatment of diabetes (Habicht et al. 2014, Hamdan et al. 2019, Mitra et al. 2019). Evidence of their inhibitory effects on carbohydrate metabolizing enzymes supports the traditional use of these plants and provides basis to isolated active phytoconstituents that can be used for clinical management of diabetes after thorough investigations.

4.4.3. Cytotoxic assays

Brine shrimp lethality assay is a well-established tool for cytotoxicity evaluation (Hamidi et al. 2014). It can be used to determine the toxicity of plant extracts as a primary indicator for their potential to be tested in cancer cell models and to

illustrate the tendency of their biological action using a zoological organism (Nguta et al. 2012). In the present study, all extracts showed significant cytotoxicity against brine shrimps with LC₅₀ values between 48.2 and 96.9 µg/ml. It implies that first, these extracts can be tested and used for biological profile at concentrations lower than this to avoid toxicity of extracts and second, their cytotoxicity must be further evaluated in normal cells like lymphocytes or fibroblasts. Previous data reported that majority of the selected plant extracts (*M. koenigii*, *N. sativa*, *C. amada*, *T. chebula* and *M. koenigii*) displayed well defined *in vitro* anticancer activity highlighting the importance of cytotoxicity re-appraisal in normal versus cancer cells (Shrestha 2017, Nerdy et al. 2021, Hossain et al. 2020, Eshwarappa et al. 2016).

In line with above data, we next determined the effect of selected extracts on protein kinase activity in *Streptomyces 85E*. The purpose was to assess the potential inhibitory effect of extracts on protein kinase enzymes that are primary regulators of carcinogenesis. Genetic mutation in cell cycle, differentiation and cellular death processes upraise protein kinase activity at serine/threonine or tyrosine levels that are frequently detected in cancers (Fatima et al. 2022a, Montagnani and Stecca 2019). Hence, the research on drug candidates inhibiting protein kinases is ongoing for the targeted treatment of cancer (Evan and Vousden 2001). *Streptomyces* involves protein kinase effect of protein RamC to transform pre-Sap B to Sap B, a surfactant necessary for the formation of aerial hyphae (Waseem et al. 2017). Inhibition of RamC creates bald zones around sample discs corresponding to the inhibition of aerial hyphae formation. All extracts had mild to moderate protein kinase inhibition activity, suggesting dose adjustment and evaluation in specific kinase systems to further appraise the cytotoxic potential of tested samples. Since it will be beneficial in understanding their potential role as antitumor and anti-infective agents (Sharma et al. 2016).

4.4.4. Biocompatibility evaluation

Hemolytic analysis explores the biocompatibility of selected extracts with human normal RBCs to illustrate the cytotoxicity mechanism to cellular membrane. This was done to ensure the safety of the extracts at concentrations where significant biological activities were observed. (Alonso and Alonso 2016). Almost all the extracts displayed less than 3 % hemolysis at 25 µg/ml. Likewise, most of the extracts showed < 10 % hemolysis at 100 µg/ml. The 200 µg/ml concentration can be considered the maximum number of extracts that can be used for biological activities to prevent further damage to RBCs. Thus, further research is planned to test the biological activities of selected extracts preferably at < 100 µg/ml concentrations to ensure their safety to normal cells. Phytochemicals may influence the membrane stability of RBCs depending on their antioxidant profile as well as possible interaction of specific functional groups with cell membranes (Rubnawaz et al. 2021). Phytochemicals can have dual effect on RBC integrity based on concentration. Literature shows that higher concentration of tannic acid can cause crinkling and lysis of RBC (Deng et al. 2019). On the contrary, phytochemicals like polyphenolic compounds, being potent antioxidants have capacity to reduce oxidative stress mediated hemolysis. It has been reported that ROS particularly thiol and peroxides cause RBC membrane rigidity and fragility leading to hemolysis

(Diederich et al. 2018). There is evidence of protection of RBCs from hemolysis by polyphenols due to their peroxidase and other free radical scavenging properties (Gwozdziński et al. 2021). Although, along with the results of brine shrimp assay, the extracts showed minor toxicity in *in vitro* assays; however, it is necessary to evaluate their interaction with the biological system in *in vivo* models. Moreover, a range of sample concentrations, methanol extracts in particular, can also be assessed in cancer cell models to evaluate their prospective activity in cancer.

4.4.5. Antimicrobial potential

Treatment of infections using herbal remedies is a common practice. These contain a variety of phytochemicals that can halt the growth of bacteria or fungi depending on specific plant part and specific organism (Kaushik and Goyal 2008). Thus, traditionally used plants are being investigated progressively for the development of safe and effective antimicrobial formulations (Sukanya et al. 2009). Methanol and aqueous extracts are distinctively active against strains of *S. aureus* and *K. pneumoniae*, consequently indicating the presence of antibacterial phytochemicals. Research showed that phenolics and flavonoids such as caffeic acid, gallic acid, rutin and apigenin have well-established antibacterial activity (Sen et al. 2016). The present study reported significant amounts of TPC and TFC in plant extracts, which is supported by previous data that showed the presence of caffeic acid, gallic acid, rutin and apigenin in extracts of *N. sativa*, *C. recutita*, *C. amada* and *T. chebula* (Al-Khalifa et al. 2021, Rao et al. 2008, Mandeville and Cock 2018). Antibacterial activity of these polyphenolic compounds involves adherence, biofilm formation, hindrance in motility and influence on metabolic enzymes of bacteria (Kahkeshani et al. 2019). One study applied *N. sativa* seed extract topically on staphylococcal skin infection in infants and observed that the infection recovery was equivalent to standard mupirocin (Rafati et al. 2014). Additionally, in a controlled clinical study, seventy-eight patients were given mouth rinse of *T. chebula* extract, which significantly reduced gingival irritation and microbial plaque in comparison to 0.12 % chlorhexidine. This correlates with our results that showed significant antibacterial activity of *C. amada* and *T. chebula* laying foundation for their use in clinical practice.

Furthermore, the antifungal potential correlates with presence of tannins, flavonoid, unsaturated lactones, phenolic compounds and essential oils (Cowan 1999). Plant's antifungal mechanism may be accredited to the flavonoid content as these can create complex with extracellular protein components present in fungi's cell wall. Likewise, flavonoids can interrupt the fungal membrane because of high degree lipophilicity (Arif et al. 2009) and interfere with efflux pumps, protein synthesis and mitochondrial function (Al Aboody and Mickymaray 2020). The mild antifungal potential of the extracts in present study can be linked with the presence of phenolic compounds that are confirmed by phytochemical analysis.

5. Conclusions

Concisely, this study reported the effect of using solvents with different polarity for extraction on the biological profile of resultant extracts. Here, the methanol extracts contained the significant phenolic and flavonoid content. These corresponded with their significant antioxidant and antibacterial activities as well as mild to moderate protein kinase

inhibition and antihyperglycemic potentials. *Nigella sativa* methanol extract showed maximum TPC. *M. longifolia* and *G. lutea* methanol extracts revealed notable antibacterial activity against *S. aureus* and *K. pneumoniae*. Similarly, methanol extracts of *C. recutita* and *M. koenigii* had significant α -glucosidase activity. Considering the protein kinase inhibitory and cytotoxic activities, all extracts are planned to be re-tested in normal versus cancer models at variable concentrations for their potential evaluation as anticancer agents. All these activities provide evidence for the traditional use of selected plants by herbalists. Future studies are in process for the qualitative and quantitative analysis, isolation, and characterization of individual bioactive phytochemicals.

CRedit authorship contribution statement

Sania Atta: Writing – original draft, Methodology. **Durdana Waseem:** Writing – review & editing. **Iffat Naz:** Writing – review & editing. **Faisal Rasheed:** Project administration. **Abdul Rehman Phull:** Data curation, Visualization. **Tofeeq Ur-Rehman:** Data curation, Visualization. **Nadeem Irshad:** Supervision, Investigation. **Parveen Amina:** Project administration. **Humaira Fatima:** Supervision, Investigation.

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