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Comparative study of ultrasonic-assisted extracts of twelve traditional medicinal plants for *in-vitro* assessment of anticancer activity along with phytochemical and antioxidant potential

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Abstract: **Background**: Pakistan has 6,000 plant species and several of these are known to have medicinal properties. However, dozens of plants are still scientifically unexplored or under-explored for their medicinal and therapeutic potential. Therefore, in the present study, twelve folk medicinal plants, including *Plantago major, Cissampelos pareira, Justicia adhatoda, Myrsine africana, Rubus fruticosus, Santalum album, Solanum pseudocapsicum, Fumaria indica, Moringa oleifera, Quercus infectoria, Flacourtia jangomas* and *Acacia karroo,* were chosen. **Objectives**: The study aimed to assess phytochemical content, antioxidant activity and anticancer potential. **Methods**: The extracts were prepared in 80% methanol under ultrasonic assistance. The antioxidant activity was measured using the DPPH method and the phytochemical content was evaluated following standard protocols. The cytotoxic potential was assessed using the MTT colorimetric assay against the HeLa cell line and multivariate regression analysis identified the common group (s) of bioactive metabolites among the studied plants. **Results**: The extraction yield was between 6.0% and 14.6%, depending on the plant material. Phytochemical analysis revealed varying levels of phenolics, flavonoids, tannins and alkaloids. Among all tested plants, *M. africana* exhibited the highest DPPH free radical-scavenging activity at 68.3 ± 1.2%, just below ascorbic acid (75.9 ± 1.5%), while *J. adhatoda* had the lowest (42.3 ± 2.5%). The MTT assay indicated that all plant extracts, except *J. adhatoda, F. indica* and *F. jangomas,* demonstrated promising anticancer effects, with *M. africana* showing the most potent activity (IC_{50} = 37.7 ± 2.4 µg/mL). The two-way orthogonal PLS multivariate regression analysis identified phenolics and flavonoids as important classes of phytochemicals present in *M. africana, S. album* and *M. oleifera,* responsible for their potent antioxidant and cytotoxic properties. **Conclusion**: The results reveal that among the studied plants, *M. africana* exhibits the most significant antioxidant and cytotoxic potential. However, further research is recommended to isolate and identify the specific antioxidant and anticancer compounds.

Keywords: Antioxidant activity; Cytotoxic activity; Medicinal plants; Phytochemicals; Principal component analysis

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INTRODUCTION

Since ancient times, man has relied on nature for food, clothing, fragrances, flavors, shelter and medicines (Gurib-Fakim, 2006). Medicinal plants, rooted in centuries of empirical knowledge, offer cost-effective, evidence-based and safe preventive healthcare compared to allopathic medicines (Bibi *et al*., 2011; Hussain *et al*., 2013; Joshi *et al*., 2011). Recent years have witnessed an expanding appreciation of their utility, with over 50% of modern clinical drugs originating from natural sources (Adnan *et al*., 2014). According to the World Health Organization, 80% of the global population, predominantly in developing nations, relies on plant-derived medicines for healthcare (Saleem *et al*., 2001; Yadav and Agarwala, 2011).

The plant contains a complex mixture of bioactive metabolites that not only support growth and protection but can also act as symbiotic agents to improve human health, functioning as antioxidants, antimicrobials, antidiabetics, anticancer agents, immune stimulators, enzyme detoxifiers, etc. (Rao., 2003). Antioxidant constituents in plants play an essential role in maintaining human health by scavenging free radicals released in the body during metabolism (Fazal *et al*., 2011). These free radicals cause many human disorders, such as arthritis, damage to the central nervous system and cancer (Ilahi *et al*., 2013; Lee

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et al., 2003). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been reported to cause several side effects. Hence, researchers today explore medicinal plants for natural antioxidant compounds, such as flavonoids, phenols and vitamins A, C and E, which are helpful in many diseases (Mathew *et al*., 2012). Cancer is considered the most alarming disease (Singh *et al*., 2018). After cardiovascular disorders, it is the second leading cause of death worldwide (Desai *et al*., 2008; Bouyahya *et al*., 2020). Cancer is characterized by the uncontrolled multiplication of tumor cells, which then spreads evenly throughout the body (Solowey *et al*., 2014). In pursuing cancer treatments with reduced toxicity and fewer side effects than conventional chemotherapy, scientific research has increasingly focused on drugs derived from natural sources, including plants with proven anticancer secondary metabolites such as phenolics, alkaloids and tannins (Greenwell and Rahman, 2015). The plant-derived medicines are based on the experimental findings of hundreds and thousands of years, which is why medicinal plants provide those preventions that are economical and comparatively safe compared to those of allopathic medicines (Bibi *et al*., 2011; Hussain *et al*., 2013; Joshi *et al*., 2011). In response to the great struggle for curing diseases, knowledge of medicinal plant usage has increased in recent years because man has succeeded in seeking drugs in different parts of plants (Fawole *et al*., 2009). As an estimate, over 50% of modern clinical medicines have a natural product origin (Adnan *et al*., 2014). According to a WHO report, 80% of the world's population, fundamentally those of developing countries, rely on plant-derived medicines for their healthcare (Saleem *et al*., 2001; Yadav and Agarwala, 2011).

The earth carries about 265,000 species of plants (among these, 3,000 plant species possess anticancer activity, but only half of these have yet been examined for their medicinal importance and chemical composition (Solowey *et al*., 2014; Sharif *et al*., 2018). Pakistan has an altitude ranging from 0-8611 meters and has very different climate zones with versatile biodiversity. Pakistan is blessed with 6,000 floral species, of which almost half have been reported from northern areas. A number of these are reported to be helpful in treating many diseases, but dozens of plants remain underexplored for their pharmacological potential (Shinwari, 2010).

Twelve underexplored or unexplored traditional medicinal plants were selected for the present research. These include: *Plantago major*, a perennial herb found in many places around the globe, which exhibits various therapeutic properties such as antiviral, anti-inflammatory and antimicrobial effects and is also equally effective in healing wounds (Zhakipbekov *et al*., 2023). *Cissampelos pareira* has been used in traditional medicine in Asia, Latin America and Africa. It is useful in female reproductive health, gastrointestinal and urinary disorders and inflammations. Some methodical investigations also reveal its strong antimicrobial, anti-inflammatory, antimalarial, analgesic, antivenom and cytotoxic potential, supported by its bioactive metabolites like alkaloids (Thim-Uam *et al*., 2025). *Justicia adhatoda* has a widespread topographical distribution and is well known for its medicinal value. It is not only effective in respiratory disorders, malaria, dysentery and tuberculosis, but also has antimicrobial and anticancerous properties (Isha *et al*., 2025). Next, *Myrsine africana* is primarily found in tropical and subtropical regions of Asia and Africa. It possesses aromatic properties and is traditionally used as a spice and flavoring agent, tea, appetizers and carminatives (Moges *et al*., 2025). *Rubus fruticosus* (common blackberry) is a wild fruit that has also been called a functional food due to its excellent nutritional profile. These berries are also reported to have various folk medicinal uses, like anti-inflammatory, anti-hemorrhoidal, anti-diarrheal, diuretic and anxiolytic effects (Cadi *et al*., 2021). *Santalum album* (Sandalwood) is a celebrated component of cosmetics, perfumes, food products and chewing tobacco. Sandalwood oil is traditionally known for antioxidant, antiviral, antifungal, antipyretic, anti-inflammatory and anticancer properties. Besides these, Aromatherapy is an important medicinal use of sandalwood (Chavan *et al*., 2024; Khan *et al*., 2021). *Solanum pseudocapsicum* is a bushy shrub traditionally known for its antimicrobial, antiviral, antihypertensive and antispasmodic activities (Singh *et al*., 2024). *Fumaria indica* is also a prominent ethnomedicinal plant, traditionally used to treat cardiovascular disorders, specifically hypertension and has momentous healing efficacy among many native communities in various regions globally (Shah *et al*., 2024).

Moringa oleifera is also known as the miracle tree due to its substantial nutritional value; it has potential for functional foods and nutraceuticals because of its richness in vitamins, proteins, carbohydrates and minerals. Its various parts (i.e., flowers, leaves, pods and seeds possess numerous important metabolites useful in the cosmetic, food and pharmaceutical industries (Gharsallah *et al*., 2023). *Quercus infectoria* is recognized for its various therapeutic applications, especially its bark, which contains compounds with antioxidant, antimicrobial and anti-inflammatory properties (Aziz and Al Kaabi, 2024). Similarly, the edible fruit-bearing plant *Flacourtia jangomas* is used in traditional medicine for several ailments, including gastrointestinal issues, infections, inflammation, diarrhea, fever, asthma, liver problems, nausea, diabetes, jaundice and biliousness (Ripa *et al*., 2024). Lastly, *Acacia karroo* is not as well-known as a medicinal plant as the others selected for this study. However, some literature shows it possesses phytochemicals like alkaloids, antioxidants, terpenoids and tannins and its extracts exhibit antioxidant and enzyme inhibitory properties (Anwar *et al*., 2024).

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Although these plants have been used for decades in various indigenous medicines in one way or another, most have not been scientifically evaluated, particularly for their anticancer potential. Therefore, the present study aimed to assess their cytotoxic potential, supported by phytochemical and antioxidant properties.

MATERIALS AND METHODS

Chemicals and reagents

The analytical grade solvents, methanol, ethanol, dimethyl sulfoxide (DMSO) and ether and the chemicals including $AlCl_3$, Na_2CO_3, NH_4OH, KH_2PO_4, CH_3COOH, Folin Ciocalteu (FC) reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and dye; 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. The standards, like Ascorbic acid, Gallic acid, Quercetin and Doxorubicin, were obtained from Sigma-Aldrich and Solarbio Life Sciences. The H1 HeLa (ATCC, Cat # CRL-1958) was acquired from the National Centre of Excellence in Molecular Biology (University of the Punjab, Lahore, Pakistan). Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute (RPMI-1640), supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) Penicillin-Streptomycin, were purchased from Gibco (Grand Island, NY).

Plant materials

The different parts of the twelve medicinal plants, *P. major, C. pareira, J. adhatoda, M. Africana, R. fruticosus, S. album, S. pseudocapsicum, F. indica, M. oleifera, Q. infectoria, F. jangomas* and *A. karroo,* were used in the present research. The plant materials were collected, identified and provided by MAPICC (Medicinal and Aromatic Plants Introduction and Conservation Center), Azad Jammu and Kashmir, Pakistan and from Mashmoom Industries Pvt Limited, Hattar Industrial Estate, KPK, Pakistan. The plant materials were packed in kraft paper and moved to the Food Science Laboratory at the University of Haripur, initially washed with tap water and dried in the shade for three weeks. (Hussain *et al*., 2011; Bibi *et al*., 2011). The dried plant material was ground into powder and sieved with a 120-micron mesh. Particles smaller than 0.5 mm are ideal for effective extraction because the solvent can properly contact the target analyte (Azwanida, 2015). The powdered samples were sealed in airtight containers and stored at -4 °C.

Preparation of extracts

50 g of each powdered plant sample was soaked in 500 mL of 80% methanol in separate volumetric flasks for 30 min. The flasks were then placed in an ultrasonic water bath (BIOBASE China) at a power of 240 W and a frequency of 40 Hz, respectively, for 60 min at a constant temperature (40 °C). The extract was filtered using Whatman No. 1 filter paper and the filtrate was subjected to drying in a rotary vacuum evaporator (Heidolph, Germany) at 40 ºC. The dense (semi-solid) extracts were further dried in a freeze-drier (Alpha 1-2 LD plus, Crist, Germany) at -50 °C. The percentage yield of each extract was calculated with the following formula (Equation 1), and the dried extracts were stored in the freezer (DW-40L262, Haier Biomedical, China) at -40 ˚C before further analysis (Ratananikom and Premprayoon, 2022, Zhang *et al*., 2011).

$$\% \text{ Yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of the plant material used}} \times 100 \quad \text{(Equation 1)}$$

Antioxidant activity

The antioxidant activity of plant samples was determined based on the ability of the extract to scavenge stable radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH). Firstly, 3.96 mg of DPPH was dissolved in 20 mL of methanol to make a stock solution. Then, 250 µL of the methanolic solution of each plant extract (250 µg/mL) was mixed with 500 µL of the DPPH solution in a 96-well plate. Ascorbic acid was used as a positive control and a blank solution was prepared similarly by adding methanol in place of the plant extract, used as a negative control. The plate was kept in the dark at room temperature for about 30 minutes and the absorbance was measured at 517 nm using a spectrophotometer. Lesser absorbance of the test sample mixture indicates higher antioxidant activity. Percent antioxidant activity was calculated with the following equation (Equation 2) (Fazal *et al*., 2011; Ali *et al*., 2026).

$$\% \text{ Antioxidant acticity} = 100 \times 1 - \frac{\text{Abs.of sample}}{\text{Abs.of blank}} \quad \text{(Equation 2)}$$

Phytochemical investigation

The medicinal properties of plants are due to phytochemical constituents (like phenolics, flavonoids, tannins, terpenoids and alkaloids) in their different parts (Shaikh and Patil, 2020). Therefore, the following quantitative analyses were performed to determine the phytochemical composition of the plant samples under study.

Determination of total phenolics

The total phenolic contents (TPC) were assessed with the Folin-Ciocalteu (FC) reagent, repeating a previously reported method (Chandra *et al*., 2014). The absorbance was obtained at 765 nm. The phenolic concentration was determined by drawing a Gallic acid standard calibration curve and represented as Gallic acid equivalent (mg GAE/g) (Chandra *et al*., 2014).

Determination of total flavonoids

Total flavonoid determination by the $AlCl_3$ assay method. Quercetin was used as a standard and a calibration curve was drawn by its serial dilutions (5-200 µg/mL). The absorbance was measured at 420 nm. The total flavonoid content was expressed as the concentration of flavonoids in mg/g of quercetin equivalent (mg QRC/g) of dried plant material (Chandra *et al*., 2014; Ghoneim *et al*. 2016).

Determination of total tannins

The Folin-Ciocalteu (FC) assay method was applied to estimate total tannin content (Mohammed and Manan, 2015). The absorbance was taken at 725 nm. Solutions of

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various concentrations (from 0-100 mg) of Catechin (CTN) standard were used to draw a calibration curve. The amount of tannins was expressed as Catechin equivalent (mg CTN/g of dry plant material).

Determination of alkaloids

Alkaloid determination was performed using the Harborne method (also known as the standard gravimetric method). A 5 g powder sample was placed in a 250 mL beaker and 200 mL of 10% CH_3COOH in ethanol was added. The mixture was covered and allowed to stand for about 4 hours, then filtered and concentrated to one-fourth of its original volume. Next, concentrated NH_4OH was added dropwise to the extract until precipitation was complete. The solution was allowed to settle; the precipitates were collected, washed with dilute NH_4OH and finally filtered. The filtrate was dried and the alkaloid content was calculated in mg/g of the sample (Mujeeb *et al.*, 2014; Daben *et al.*, 2021; Hosseinzadeh Rostam Kalaei *et al.*, 2022).

Anticancer activity

Cell culture

The HeLa cells were cultured in DMEM and RPMI-1640 supplemented with 10% (v/v) Fetal Bovine Serum and 1% (v/v) Penicillin-Streptomycin (Gibco) in a humidified incubator at 37 ºC with 5% CO_2 and 95% air and the medium was changed every other day (Zakki *et al*., 2020).

Cell viability analysis

To perform the assay, cultured cells were seeded in a 96-well microplate at 3.5×103 cells per well and incubated in a 5% CO_2 incubator at 37 °C for 48 hr. Then, different plant extracts (dissolved in DMSO ≤1 %) at concentrations of 0-200 µg/mL were added at 20 µl/well. A separate plate (Control) was prepared with 20 µl/well of DMSO (≤1 %) instead of plant extracts. The plates were further incubated at 37 °C for 24 hr. in a 5% CO2 incubator. Then, 20 µl of sterile MTT (2 mg/mL) dissolved in phosphate-buffered saline (PBS) was added to each well. The plates were covered with aluminum foil and incubated for about 3-4 hr. under the same conditions. The mitochondrial dehydrogenase enzyme reduced the tetrazolium salt to a formazan dye. This conversion reflects the normal function of the cellular enzyme and proportionally represents the number of viable cells. The insoluble formazan crystals were then solubilized by adding DMSO (about 100 µl, ≤1 %). The formazan product was spectrophotometrically quantified by measuring absorbance at 540 nm using BMG LABTECH's innovative absorbance microplate reader. Then, the treated and untreated plates were compared and the percentage cell viability was calculated using the following formula (Equation 3) (Ala *et al*., 2018; Batool *et al*., 2021; Ali *et al*., 2026).

$$\% \text{ Cell viability} = \frac{\text{Abs.of sample} - \text{Abs.of blank}}{\text{Abs.of control}} \times 100 \text{ (Equation 3)}$$

Statistical analysis

All the analytical calculations were performed in triplicate and the results are presented as the mean ± standard deviation (SD). For cytotoxic activity, the least significant difference (LSD) on mean values and analysis of variance (ANOVA) were tested using one-way ANOVA with GraphPad Prism (GraphPad Prism 6.0 Software, San Diego, CA). To establish correlations among phytochemical and antioxidant activity values and the reciprocals of IC_{50} values for cytotoxic activity, multivariate data analysis (MVA) was performed using SIMCA-P software (v. 11.0, Umetrics, Umeå, Sweden).

RESULTS

Plant species and yield of extracts

The results (Table 1) show that different plant parts used in the study yielded variable amounts of extract. The maximum yield was obtained for *J. adhatoda* (14.6%), while *Q. infectoria* showed the lowest yield (6.0%) among all the selected plant extracts.

Antioxidant activity

The results obtained (Table 2) provided valuable insights into the antioxidant activity of different plant materials. As a reference, ascorbic acid, a well-established standard, demonstrated a robust antioxidant activity of 75.9 ± 1.5%, whereas the control group exhibited minimal antioxidant activity (0.4 ± 0.1%). The investigation of various plant samples revealed a spectrum of antioxidant profiles. Notably, *M. africana* displayed the highest antioxidant activity (68.3 ± 1.2%) among all plant samples. Similarly, *S. pseudocapsicum* and *M. oleifera* also demonstrated noteworthy antioxidant activity, i.e., 67.2 ± 2.1% and 66.3 ± 2.4%, respectively. On the other hand, *J. adhatoda* exhibited the lowest antioxidant (42.3 ± 2.5%) effect among the tested samples.

Phytochemical analysis

Total phenolics

The results (Table 3) indicate that the *S. album* extract displayed the highest phenolic content, 17.2 mg GAE/g, followed by *C. pareira* (16.8 mg GAE/g) and *A. Karroo* (15.93 mg GAE/g). Conversely, the *J. adhatoda* leaf extract exhibited the lowest phenolic content among the samples, measuring only 3.8 mg GAE/g.

Total flavonoids

The results (Table 3) show that the *S. album* extract exhibits the highest flavonoid content (17.1 ± 2.0 mg QRC/g), indicating the presence of substantial flavonoid compounds. In contrast, the *J. adhatoda* extract has the lowest flavonoid content (0.5 ± 0.1 mg QRC/g). Among the other plant extracts, *M. africana*, *R. fruticosus* and *P. major* demonstrated notable flavonoid contents, i.e., 15.1 ± 1.7 mg/g, 11.5 ± 1.0 mg/g and 10.9 ± 0.2 mg QRC/g, respectively. Conversely, the remaining plant samples contained less than 10 mg/g QRC of flavonoids.

Total tannins

Tannins are natural polyphenolic compounds known for their antioxidant properties (Tong *et al*., 2021).

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Table 1: Botanical name, local name/s, family, part of the plant used and the percentage yield of extracts of medicinal plants under study.

Plant species (Botanical name)	Local name	Family	Part used	Yield of extract (%)
Plantago major	Bartang	Plantaginaceae	Whole plant	11.3
Cissampelos pareira	Patha	Menispermaceae	Stems	9.7
Justicia adhatoda	Bhaiker, Bansa	Acanthaceae	Leaves	14.6
Myrsine africana	Khukan	Myrsinaceae	Leaves	7.8
Rubus fruticosus	N/A	Rosaceae	Roots	6.2
Santalum album	White Sandal	Santalaceae	Bark	10.8
Fumaria indica	Shahtra, Papra	Fumariaceae	Whole plant	12.7
Moringa oleifera	Sohanjna	Moringaceae	Seeds	8.2
Quercus infectoria	Mazu Sabz	Fagaceae	Seeds	6.0
Flacourtia jangomas	Zarnab	Salicaceae	Stems	10.2
Acacia Karroo	Chaal Kikar	Fabaceae	Bark	13.4
Solanum pseudocapsicum	N/A	Solanaceae	Whole plant	13.5

Table 2: Antioxidant activity of plant extracts under study.

Extracts	Antioxidant activity (%)	Extracts	Antioxidant activity (%)
Plantago major	55.3 ± 3.1	*Moringa oleifera*	66.3 ± 2.4
Cissampelos pareira	56.1 ± 2.8	*Quercus infectoria*	59.1 ± 3.4
Justicia adhatoda	42.3 ± 2.5	*Flacourtia jangomas*	52.2 ± 0.9
Myrsine africana	68.3 ± 1.2	*Acacia Karroo*	61.2 ± 1.3
Rubus fruticosus	60.0 ± 0.9	*Solanum pseudocapsicum*	67.2 ± 2.1
Santalum album	66.1 ± 2.9	Standard (Ascorbic acid)	75.9 ± 1.5
Fumaria indica	59.2 ± 1.6	Blank	0.4 ± 0.1

Table 3: Different phytochemical constituents of the plant extracts under study.

Extract	Total phenolics mg GAE/g	Total flavonoid mg QRC/g	Total tannins mg CTN/g	Total alkaloid mg/g Ext.
Plantago major	7.0 ± 0.4	10.9 ± 0.2	11.4 ± 1.1	1.2 ± 0.2
Cissampelos pareira	16.8 ± 0.8	8.1 ± 0.5	16.0 ± 1.8	3.0 ± 0.4
Justicia adhatoda	3.8 ± 0.1	0.5 ± 0.1	8.2 ± 0.3	2.6 ± 0.4
Myrsine africana	11.1 ± 1.6	15.1 ± 1.7	13.0 ± 1.4	1.5 ± 0.2
Rubus fruticosus	4.9 ± 1.3	11.5 ± 1.0	12.9 ± 1.9	1.9 ± 0.3
Santalum album	17.2 ± 0.1	17.1 ± 2.0	14.1 ± 1.9	1.1 ± 0.2
Fumaria indica	6.3 ± 0.9	3.2 ± 0.2	8.2 ± 0.4	2.8 ± 0.1
Moringa oleifera	8.5 ± 1.5	5.7 ± 0.9	8.9 ± 1.1	2.3 ± 0.2
Quercus infectoria	14.1 ± 1.8	4.8 ± 0.4	19.8 ± 0.3	3.2 ± 0.3
Flacourtia jangomas	6.7 ± 1.5	2.9 ± 0.6	10.6 ± 3.0	3.4 ± 2.2
Acacia Karroo	15.9 ± 1.7	10.4 ± 2.2	15.1 ± 2.5	3.8 ± 0.9
Solanum pseudocapsicum	14.2 ± 1.7	6.0 ± 0.2	16.2 ± 1.1	1.9 ± 0.2

Total alkaloids

The results (Table 3) indicate that the *A. karroo* bark extract had the highest alkaloid concentration, i.e., 3.8 ± 0.9 mg per gram of extract (mg/g Ext.). On the other hand, the lowest alkaloid concentration (1.1 ± 0.2 mg/g Ext.) was shown for the *S. album* and *P. major* extracts. The remaining plant extracts under study also hold a considerable amount of alkaloids. Generally, the quantity of alkaloids in all samples was significantly less than the other assayed phytochemicals. The *S. album* bark extract had the highest tannin content (12.9 ± 1.9 mg CTN/g), while *J. adhatoda* (8.2 ± 0.3 mg CTN/g) and *F. indica* (8.2 ± 0.4 mg CTN/g) both had the lowest tannin content (Table 3). The overall findings indicate relatively lower tannin concentrations in these particular extracts compared to others. However, notable amounts of tannins were observed in the remaining evaluated extracts.

Anticancer activity

The selected plant extracts were *in-vitro* assessed for cytotoxic potential by the MTT assay method (Koul *et al*., 2015). Figure 1 shows the effect of different plant extracts (in various concentrations) on the cell viability of the HeLa

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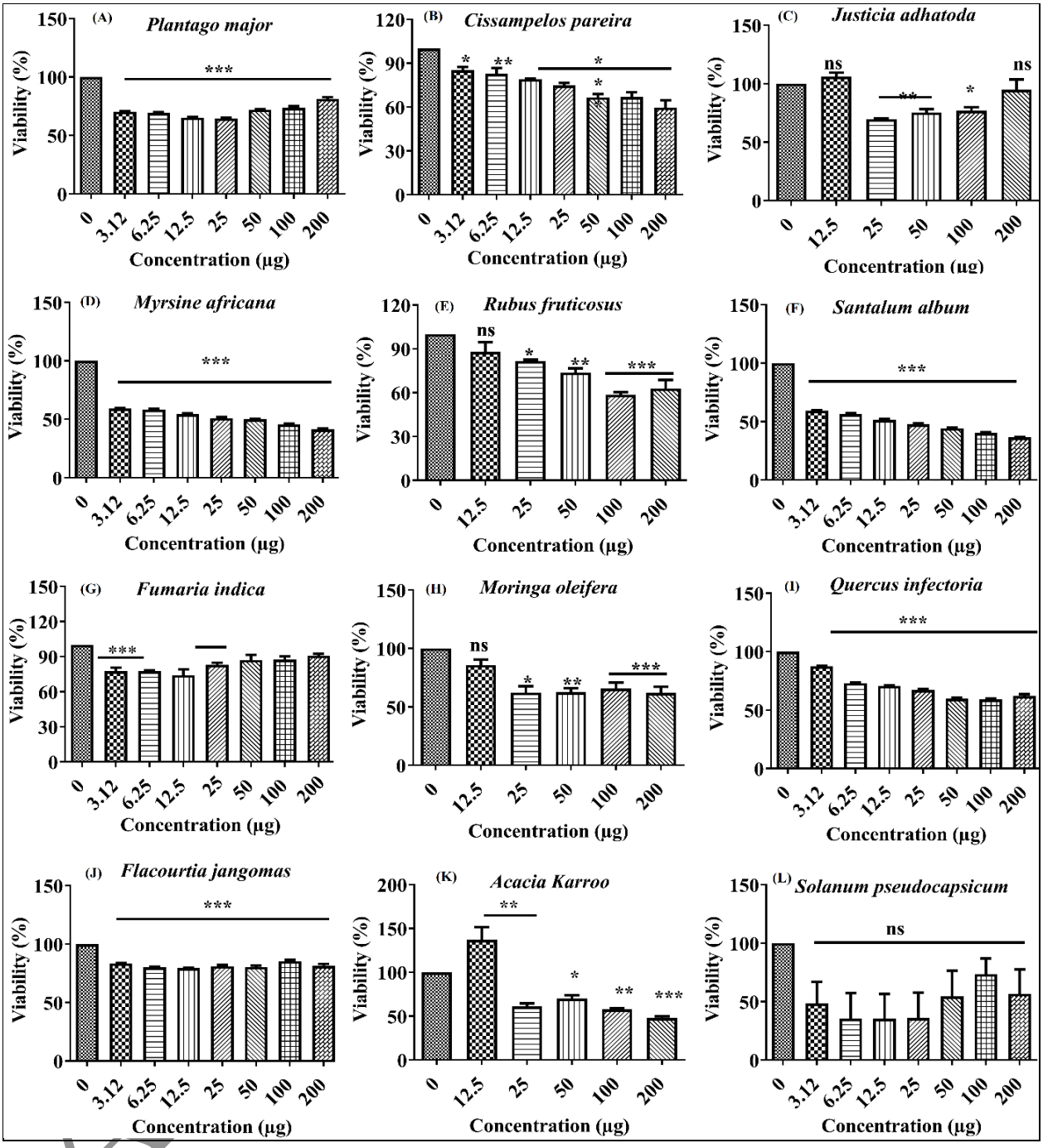


Fig. 1: Effect of different plant extracts on cell viability in the HeLa cancer cell line. The cells were treated with the indicated concentrations of plant extracts for 48 h and MTT assays were used to test the viability. *p < 0.05, **p < 0.01 and ***p < 0.001 vs. the control group.

cell line and table 4 represents the IC_{50} values for the cytotoxic activity of the evaluated samples.

The results (Fig. 1) reveal that about half of the tested plant extracts, i.e., *C. pareira* (B), *M. Africana* (D), *R. fruticosus* (E), *S. album* (F) and *Q. infectoria* (I), showed dose-dependent anticancer effects and significantly reduced the tumor cell viability. Whereas the three extracts, *P. major* (A), *M. oleifera* (H), *A. karroo* (K) and *S. pseudocapsicum* (L), exhibited a non-dose-dependent cytotoxic effect.

The remaining three extracts, including *J. adhatoda* (C), *F. indica* (G) and *F. jangomas* (J), did not significantly reduce the viability of the HeLa cell line under the tested conditions. Among all, *M. africana* extract (D) demonstrated the strongest cytotoxic profile, with the lowest IC_{50} value, i.e., 37.7 ± 2.4 µg/mL, close to the standard drug, Doxodubicin (IC_{50} = 11.6 ± 0.9), followed by *A. karroo* (K); IC_{50} = 74.0 ± 1.3 µg/mL and *S. album* (F); IC_{50} = 140.4 ± 3.8 µg/mL.

Table 4: Cytotoxic potential (IC_{50}) of evaluated plant extracts against the HeLa cell line.

Extract	Cytotoxicity (IC_{50} µg/mL)	Extract	Cytotoxicity (IC_{50} µg/mL)
Plantago major	681.4 ± 4.2^{pq}	*Moringa oleifera*	234.7 ± 3.6^{h}
Cissampelos pareira	401.2 ± 3.7^{m}	*Quercus infectoria*	282.5 ± 1.9^{j}
Justicia adhatoda	NA	*Flacourtia jangomas*	NA
Myrsine africana	37.7 ± 2.4^{b}	*Acacia Karroo*	74.0 ± 1.3^{cd}
Rubus fruticosus	264.5 ± 1.2^{i}	*Solanum pseudocapsicum*	265.6 ± 3.5^{i}
Santalum album	140.4 ± 3.8^{g}	Doxorubicin standard	11.6 ± 0.9^{a}
Fumaria indica	NA	Control	NA

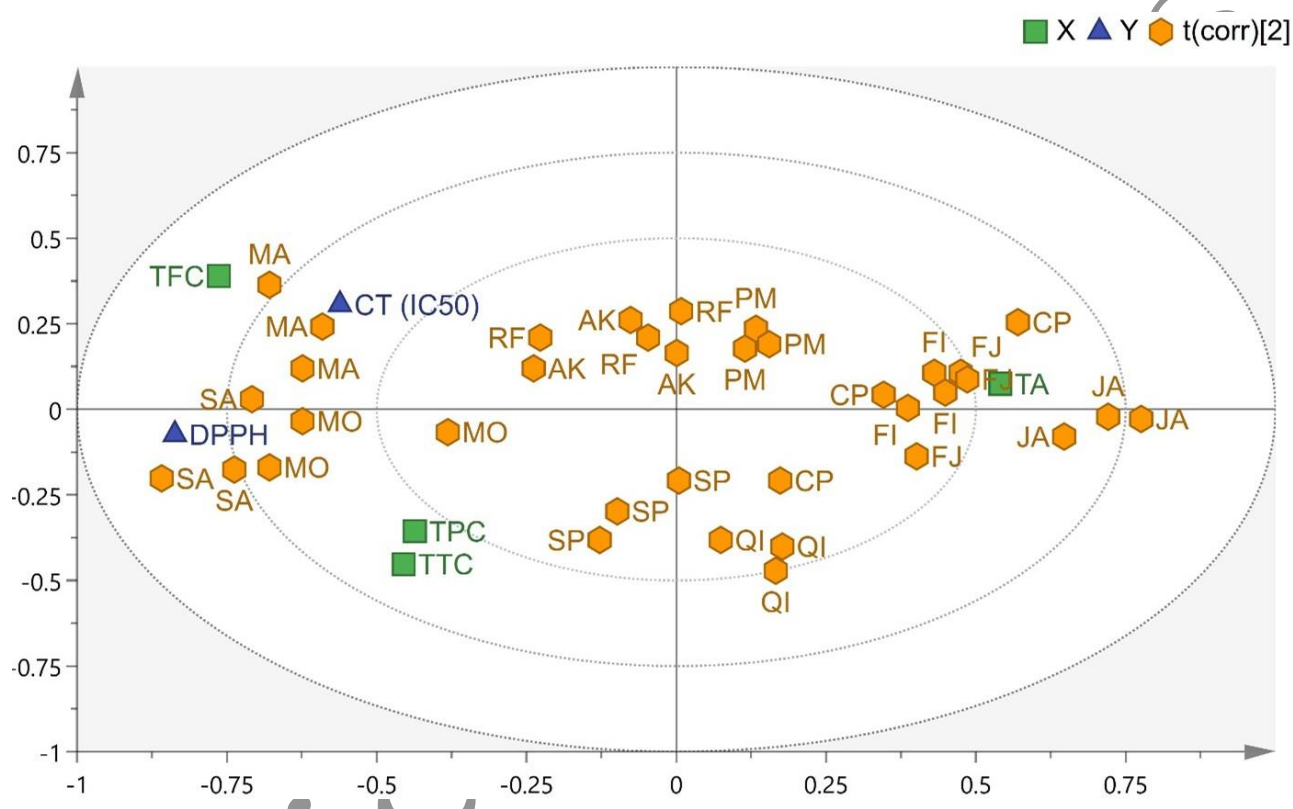


Fig. 2: Two-way orthogonal partial least square (O2PLS) Bi-Plot for antioxidant and phytochemical analysis of plant materials; DPPH = Antioxidant activity, TPC = Total Phenolic compounds, TFC = Total Flavonoid compounds, TTC = Total Tannins, TA = Total Alkaloids, PM = *P. major*, CP = *C. pareira*, JA = *J. adhatoda*, MA = *M. africana*, RF = *R. fruticosus*, SA = *S. album*, SP = *S. Pseudocapsicum*, FI = *F. indica*, MO = *M. oleifera*, QI = *Q. infectoria*, FJ = *F. jangomas*, AK = *A. karroo*

The remaining samples also showed antitumor potential with IC_{50} values not exceeding 401.2 ± 3.7 µg/mL (for *C. pareira*), except *P. major*, which showed the highest IC_{50} value, i.e., 681.4 ± 4.2 µg/mL, establishing it as the statistically least potent extract. Extracts of *Justicia adhatoda*, *Flacourtia jangomas* and *Fumaria indica* showed no activity detectable at an IC_{50} value and were omitted from final variance assessments.

Multivariate data analysis

Plants have been a significant source of bioactive compounds with various therapeutic properties, such as antioxidants, phytochemicals and anticancer agents. The complexity of plant phytochemical composition makes it challenging to understand the relationships between constituents and biological activities. To address this issue, a two-way orthogonal partial least squares (O2PLS) approach was employed as a multivariate statistical tool to reduce the dimensionality of the data and identify the patterns of correlation among plant species, as multivariate data analysis is a widely used statistical technique to simplify, discuss and interpret complex data (Jahangir *et al*., 2008, Granato *et al*., 2018). Clear discrimination has been observed using O2PLS due to the presence of different classes of phytochemicals and their relative bioactivities (i.e., CT and DPPH scavenging). The results of O2PLS were visualized in a two-dimensional Bi-plot (Fig. 2), where PLS1 explained 42.6% of the variance and PLS2 explained 13.9%.

DISCUSSION

The medicinal plants evaluated in the present study possess different biological and medicinal properties as reported in the literature. In the present work, the noteworthy free radical scavenging activity observed in *M. africana*, *S. pseudocapsicum* and *M. oleifera* extracts is likely attributable to their substantial content of phenolic compounds, flavonoids and tannins, as indicated by multivariate data analysis (Fig. 2). These secondary metabolites are renowned for their capacity to neutralize reactive oxygen species (ROS) and mitigate oxidative damage. Their electron-donating ability effectively neutralizes harmful free radicals and prevents oxidative stress-induced cellular damage (Ghoneim *et al*. 2016).

The significant variations in phenolic and flavonoid contents highlight the diversity in phytochemical composition across the studied plant extracts. This observation underscores the potential for these extracts, particularly *S. album, C. pareira, M. africana, R. fruticosus* and *P. major,* as a potent source of antioxidant compounds, which may have implications for various applications, such as in the pharmaceutical and food industries (Gurib-Fakim, 2006). Similarly, tannins are natural polyphenolic compounds known for their antioxidant properties and as antimicrobial agents in the food industry.

The noteworthy amount of tannins, especially in *S. album,* indicates its potential to act as effective food preservatives that can be used in food packaging materials, helping to preserve and maintain the quality of various food products (Tong *et al*., 2021; Singh and Kumar, 2020). Likewise, the highest content of alkaloids in *A. karroo* also indicates its potential for the extraction of natural alkaloids for the pharmaceutical and organic synthesis industry (Kurek, 2019). Literature equally supports these results; research on seven plant extracts, including *M. africana,* shows that its extract significantly inhibited the growth of the A549 cell line (Human lung carcinoma epithelial cells) and induced apoptosis, or programmed cell death, in these cells (Ahn *et al*., 2019).

The leaves of *A. karroo* and some other species of *Acacia* also possess cytotoxic potential and the results are close to the data of this study. Still, no data were found for the anticancer activity of its bark extracts (Prakash *et al*., 2021; Alajmi *et al*., 2017). Many studies demonstrate the *in-vivo* and *in-vitro* antitumor potential of various extracts of *S. album* (Yan *et al*., 2020; Santha and Dwivedi, 2015; Kumar *et al*., 2015).

Similarly, previously reported work is in strong agreement with the findings on the cytotoxicity of the studied extracts. For instance, different extracts of *C. pareira* displayed similar *in-vitro* anticancer activity against the MCF-7 cancer cell line and *in-vivo* antitumor effect on mice with induced tumors (Thavamani *et al*., 2014). The leaf extract of *M. oleifera* was equally efficient (as in the findings of the present research) against various cancer cell lines reported in different studies (Mumtaz *et al*., 2021; Barhoi *et al*., 2021; Kinga Szlachetka *et al*., 2020; Khor *et al*., 2018; Ali and Chatterton, 2018). The extract of *R. fruticosus* possessed comparable anticancer activity against breast cancer cells (Mirazi and Hosseini, 2020). *S. pseudocapsicum* and *Q. infectoria* also exhibit antitumor effects analogous to current results against various cancer cells (Hayee *et al*., 2022; Elham *et al*., 2021; Jain, 2019). The previously reported studies also reveal that *P. major* extracts exhibit antitumor effects against various cancer cell lines. Besides these, some studies have also demonstrated different results than those obtained for *J. adhatoda*, *F. indica* and *F. jangomas* in the current study (Hayee *et al*., 2022; Mello and Zancan, 2022; Parvin *et al*., 2011; Kumar *et al*., 2022). Overall, these results are in close agreement with previous findings and most of these plant extracts could be a potential source of natural compounds for developing new anticancer drugs. Still, there is a need to evaluate the plants on a larger scale to identify and isolate potential anticancer compounds (Hayee *et al*., 2022; Mello and Zancan, 2022).

The multivariate data analysis exposed intriguing correlation patterns due to metabolomic variation resulting in discrimination among plants and their bioactivities, i.e., antioxidant and anticancer activity. It is worth mentioning that, interestingly, *M. africana*, *S. album* and *M. oleifera* (Fig. 2) demonstrated discrimination in the bio-plot due to their antioxidant activity, phenolics, flavonoids and tannins relevant to antioxidant cytotoxicity. Conversely, alkaloids played a positive role in discriminating *J. adhatoda, F. jangomas, F. indica* and *C. pareira* from others in PLS-1. In contrast, antioxidant activity, phenolics, flavonoids, tannins and cytotoxicity showed negative effects. Additionally, specific observations were made regarding certain plant materials; i.e., *S. album* showed notably high antioxidant activity (66.14%). In contrast, *M. africana* exhibited higher flavonoids (15.18 mg QRC/g) and cytotoxicity (IC_{50} = 37.76 µg/mL). Furthermore, *A. karoo*, *P. major*, *R. fruticosus*, *S. pseudocapsicum* and *Q. infectoria* displayed average values across all analyses but showed a positive correlation with cytotoxicity.

CONCLUSION

The findings reveal that the studied folk medicinal plants contained varying amounts of phenolics, flavonoids, tannins, terpenoids and alkaloids. Most of these plants showed substantial antioxidant activities and some also exhibited significant anticancer potential. Such as, *Myrsine africana*, *Santalum album* and *Moringa oleifera* and multivariate data analysis suggest that these plants may share similar classes of phytochemicals (Such as phenolics, flavonoids, tannins and alkaloids) responsible for their antioxidant and cytotoxic effects. These findings

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contribute valuable insights into the chemical diversity and potential applications of these plant materials in various fields, including medicine, functional foods and nutraceuticals. However, these results were obtained in laboratory *in-vitro* experiments and further studies (such as identification and isolation of active compounds in evaluated extracts and *in-vivo* studies) are needed to determine the efficacy and safety of the active metabolomic content in humans.

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Authors' contributions
Sajjad Ali: Methodology, investigation, formal analysis, conceptualization, writing original draft; Muhammad Riaz and Muhammad Shafique: Methodology, investigation, writing original draft; Khurshid Anwar: Methodology, formal analysis; Nayab Tahir and Hina Manzoor: Investigation, writing original draft; Tahir Jamshaid and Muhammad Akram: Writing, review and editing; Shahbaz Ahmed Zakki: Validation and data analysis; Naila Bashir; Data curation and writing original draft; Huaiji Zheng: Conceptualization, writing, review and editing; Muhammad Jahangir and Inam-u-llah: Supervision, resources, project administration, review and editing.

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The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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The authors declare no conflict of interest, whether financial or personal, that could influence the publication of this study.

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