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EVALUATION OF CYTOTOXIC, ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF MEDICINAL PLANTS - BERGENIA, SANTOLINA, ARTEMISIA FROM THE FLORICULTURE DEVELOPMENT SCHEME, LAL MANDI SRINAGAR

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ABSTRACT

Medicinal plants remain an important source of bioactive compounds with potential applications in cancer prevention and therapy. In the present study, three medicinal plant extracts — Artemisia sp. (ART-2), Bergenia sp. (BEG-4), and Santolina sp. (ST-5) — collected from the Floriculture Development Scheme, Lal Mandi, Srinagar, were evaluated for antioxidant potential, in vitro cytotoxic activity against MCF-7 human breast cancer cells, and antibacterial activity against Escherichia coli. Antioxidant screening was performed using the DPPH radical-scavenging assay, cytotoxicity was assessed by the MTT assay after 24 h exposure, and antibacterial activity was assessed by the Kirby-Bauer disc diffusion method. In the MTT assay, ST-5 exhibited the strongest cytotoxic activity with an IC_{50} of 54.03 ± 0.110 µg/ml, followed by ART-2 (66.56 ± 0.091 µg/ml), while BEG-4 was the weakest (964.2 ± 0.053 µg/ml). In the DPPH assay, BEG-4 showed the strongest antioxidant activity with an IC_{50} of 33.25 ± 0.058 µg/ml, comparable to ascorbic acid (31.03 ± 0.016 µg/ml), while ART-2 (837.3 ± 0.096 µg/ml) and ST-5 (993.2 ± 0.035 µg/ml) were substantially weaker antioxidants. In the antibacterial assay, BEG-4 and ST-5 produced inhibition zones of 6 mm, while ART-2 produced 2 mm at 62.5 µg/disc, compared to ciprofloxacin (27 mm at 8 µg). The positive control paclitaxel showed an IC_{50} of 53.65 ± 0.173 nM. These findings indicate that ST-5 and ART-2 are priority candidates for further phytochemical fractionation for anticancer studies, while BEG-4 warrants investigation of its antioxidant and antibacterial constituents.

KEYWORDS: Artemisia; Bergenia; Santolina; MCF-7; MTT assay; DPPH; antioxidant; cytotoxicity; antimicrobial; Kashmir; phytochemicals

INTRODUCTION

Medicinal plants are known for their potent anticancer properties through bioactive compounds such as alkaloids, flavonoids, and polyphenols. They fight tumors by promoting cancer cell death (apoptosis), preventing new blood vessels from forming (anti-angiogenesis), and altering cell signaling. A number of modern chemotherapeutic drugs were directly derived from these botanical sources.

The strong anticancer properties of Bergenia, Santolina and Artemisia species in vitro are well known. The cytotoxicity is mainly attributed to polyphenols and terpenes which induce apoptosis, DNA damage and cell cycle arrest in different human cancer cell lines.Bergenia species exhibit promising anticancer properties in preclinical studies, mainly due to the presence of bioactive compounds such as bergenin, gallic acid and catechin. The extracts act by induction of apoptosis (programmed cell death), cell cycle arrest of cancer cells, increased oxidative stress and inhibition of tumor growth and metastasis. Plants of the genus Artemisia have strong anticancer activities against several types of cancers such as breast, lung, colorectal and liver cancers . The effects are mainly due to artemisinin and its semi-synthetic derivatives (e.g., artesunate, artemether and dihydroartemisinin) and a number of flavonoids and phenolic compounds contained in the plant extracts . The anticancer activity of extracts and essential oils of Santolina species (Santolina chamaecyparissus, Santolina pinnata) is promising in vitro and in vivo. The effects are mainly due to bioactive compounds such as phenolic compounds, flavonoids and terpenes (e.g. artemisia ketone, camphor). [1,2,13–19].

Medicinal plants are especially valuable in this context because they contain a wide variety of secondary metabolites, including phenolics, flavonoids, terpenoids, alkaloids, tannins, and glycosides. Many of these compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and antiproliferative properties. Antioxidants can reduce oxidative stress by scavenging free radicals or chelating redox-active metals, and oxidative stress is increasingly recognized as a contributing factor in cancer initiation and progression [3–5,17–19].

Artemisia is one of the largest and most diverse genera in the family Asteraceae, comprising over 500 species widely distributed across temperate regions of Asia, Europe, and North America [13,16]. The genus is particularly rich in sesquiterpene lactones, including artemisinins and their derivatives, which are well-documented as potent

cytotoxic agents capable of inducing apoptosis and cell-cycle arrest in various cancer cell lines [13–16]. Artemisia species also contain flavonoids, phenolic acids, and essential oil constituents that contribute to their antioxidant and antimicrobial activities. Published studies confirm that crude Artemisia extracts exhibit measurable antibacterial activity against both Gram-positive and Gram-negative organisms, including Escherichia coli, and display free-radical quenching capacity in DPPH and FRAP assays [14,17]. The Kashmir region harbours several Artemisia species that are used in traditional medicine for fever, inflammation, and gastrointestinal complaints, providing a strong ethnopharmacological rationale for biological screening [6–9].

Bergenia (family Saxifragaceae) is an ethnobotanically important genus whose rhizomes and leaves are used in traditional Himalayan and Unani medicine for the treatment of urinary disorders, inflammation, and infections [10,11]. The genus is chemically characterized by bergenin (a C-glycoside of 4-O-methylgallic acid), gallic acid, catechin, epicatechin, and condensed tannins — phytochemicals with well-established antioxidant, anti-inflammatory, and antibacterial properties [10,11]. Multiple studies have demonstrated that Bergenia extracts exhibit potent DPPH radical-scavenging activity attributable primarily to their high polyphenolic content. Antibacterial activity against E. coli and other pathogens has been reported for both aqueous and hydroethanolic Bergenia extracts. Cytotoxic data on cancer cell lines are more limited, but bergenin and related gallic acid derivatives have shown antiproliferative effects in certain in vitro models, warranting systematic screening [10,11].

Santolina (family Asteraceae) is a genus of aromatic shrubs native to the Mediterranean basin and traditionally used as antiseptic, antispasmodic, and antiparasitic remedies [12,22,23]. The genus is phytochemically characterized by monoterpenes, sesquiterpenes, flavonoids, and coumarins — particularly in the essential oil fraction — which are known to contribute to both antimicrobial and antiproliferative activities. Although Santolina is less studied than Artemisia and Bergenia, published reports indicate that extracts and essential oils of Santolina species display moderate antibacterial activity and cytotoxicity in cancer cell line models [12,22,23]. The antioxidant capacity of Santolina has been attributed primarily to its flavonoid and polyphenol content. The present study provides, to our knowledge, one of the first evaluations of Santolina extracts obtained from the Kashmir Floriculture Scheme in a multi-assay biological screening panel.

The Kashmir region is rich in medicinal flora, and many species are used traditionally for various therapeutic purposes [6–9]. In the present work, the three selected plant extracts were screened for antioxidant, anticancer, and antibacterial activity in order to identify promising candidates for further phytochemical investigation. Screening such plants for DPPH scavenging, MCF-7 cytotoxicity, and antibacterial potential therefore links traditional knowledge, redox biology, and anticancer prioritization in a single experimental pipeline. The present study therefore aimed to evaluate the DPPH antioxidant potential, the in vitro cytotoxic effects against MCF-7 breast cancer cells, and the antibacterial activity against E. coli of three selected Kashmiri medicinal plant extracts [1–5,10–19].

MATERIALS AND METHODS

Plant Material and Sample Coding

Three medicinal plant samples were evaluated in this study. The samples were coded as ART-2 (Artemisia sp.), BEG-4 (Bergenia sp.), and ST-5 (Santolina sp.). The plant material was collected from the Floriculture Development Scheme, Lal Mandi, Srinagar, Jammu and Kashmir, India. The specimens were botanically authenticated according to the institutional records maintained at the Floriculture Development Scheme. Herbarium voucher documentation follows the collection records at that site. Future work should confirm species-level identification and deposit voucher specimens in a recognized herbarium.

Extract Preparation

The plant material was shade-dried and ground to a coarse powder. Briefly, 20 g of each plant sample was mixed with 250 mL of 70% ethanol and stirred in a water bath at 45 °C for 10 h. The extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure at 40 °C using a rotary evaporator, lyophilized, and stored at −20 °C until analysis. Stock solutions were prepared in DMSO and further diluted to the required concentrations in the respective assay media; the final DMSO concentration in all assay wells did not exceed 0.1% v/v. Extraction yield values (% w/w, dry weight basis) were not available from the source records and should be reported in future work.

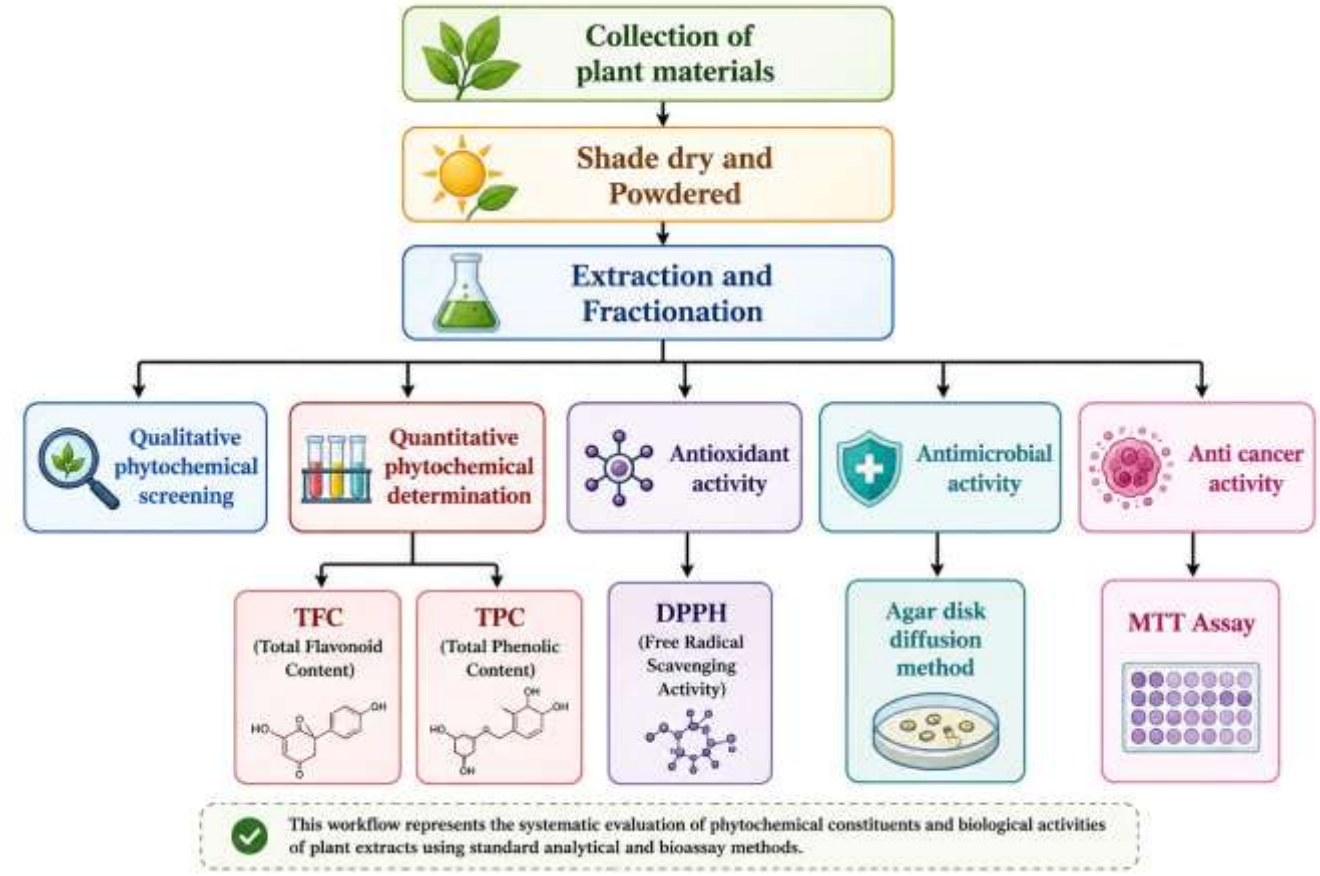

Figure 1. Experimental workflow showing plant collection, shade-drying and powdering, extraction and fractionation, followed by parallel screening for qualitative phytochemical analysis, quantitative phytochemical determination (TPC and TFC), antioxidant activity (DPPH), antibacterial activity (agar disc diffusion), and anticancer activity (MTT assay).

Antioxidant Assay (DPPH)

Antioxidant potential was assessed using the DPPH radical-scavenging assay. Fresh DPPH solution (0.3 mmol/L in methanol) was mixed with plant extract at eight concentrations (0, 1, 10, 50, 100, 250, 500, and 1000 µg/ml) and incubated in the dark at room temperature for 10 min. Absorbance was measured at 517 nm, and antioxidant activity was expressed as percentage inhibition (% RSA = $[(A_n - A_s) \div A_n] \times 100$). IC_{50} values were calculated using GraphPad Prism 6. Ascorbic acid served as the reference standard. Lower IC_{50} values indicate stronger antioxidant activity [3–5].

Antibacterial Assay (Disc Diffusion)

Antibacterial activity was assessed against Escherichia coli (MTCC-452, procured from Microbial Type Culture Collection and Gene Bank, Chandigarh) by the Kirby-Bauer disc diffusion method. Mueller-Hinton Agar (MHA) plates were inoculated with 100 µl of bacterial culture prepared at 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Whatman No. 1 filter paper discs (5 mm) loaded with 5 µl of plant extract at concentrations ranging from 0 to 200 mg/ml were placed on inoculated plates. Ciprofloxacin (8 µg/disc) served as positive control and DMSO-loaded discs served as vehicle control. Plates were incubated at 37 °C for 24 h and zones of inhibition were measured in mm [36].

Cell Culture and MTT Assay

Cytotoxicity was evaluated against the MCF-7 human breast adenocarcinoma cell line procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM (HiMedia AT149-1L) supplemented with 10% FBS (HiMedia RM10432) and 1% antibiotic solution (Penicillin-Streptomycin, Sigma-Aldrich P0781) at 37 °C in a humidified atmosphere containing 5% CO_2 (Heal Force HF90 incubator). Cells were seeded at 10,000 cells per well in 96-well plates and incubated for 24 h to allow attachment.

The medium was then replaced with incomplete medium (DMEM without FBS) containing the plant extract at concentrations of 0, 1, 10, 50, 100, 250, 500, and 1000 µg/ml. Paclitaxel (0–1000 nM) served as the positive control. Untreated cells served as control and wells without MTT served as blank. After 24 h treatment, MTT solution (5 mg/ml) was added and plates were incubated for a further 2 h. The culture supernatant was aspirated, formazan crystals were dissolved in 100 µl DMSO, and absorbance was read at 540 nm using an ELISA plate reader (iMark, Bio-Rad, USA). Cell viability was calculated as: % Viable cells = (A test / A control) × 100. IC_{50} values were calculated using GraphPad Prism 6. Results are expressed as mean ± SEM [1,2].

Statistical Analysis
All experiments were performed in triplicate (n = 3) unless otherwise stated. IC_{50} values were derived by nonlinear regression (variable-slope sigmoidal dose–response model) using GraphPad Prism 6 and reported as mean ± SEM. Comparative interpretation was based on dose–response trends and relative potency rankings among the three extracts.

RESULTS
Antioxidant Screening — DPPH Assay (Bergenia, Santolina, Artemisia)
The three plant extracts showed clear differences in DPPH radical-scavenging activity across a concentration range of 1–1000 µg/ml. BEG-4 (Bergenia sp.) displayed the strongest antioxidant effect, with an IC_{50} of 33.25 ± 0.058 µg/ml, closely approaching the value of the reference standard ascorbic acid (IC_{50} = 31.03 ± 0.016 µg/ml), indicating near-reference-level free-radical quenching capacity. ART-2 (Artemisia sp.) showed substantially weaker antioxidant activity with an IC_{50} of 837.3 ± 0.096 µg/ml, while ST-5 (Santolina sp.) was the weakest antioxidant among the three extracts with an IC_{50} of 993.2 ± 0.035 µg/ml. The rank order of antioxidant potency was therefore: ascorbic acid ≈ BEG-4 >> ART-2 > ST-5. Dose–response curves for all three extracts showed progressive inhibition with increasing concentration, confirming assay validity (R^2 values 0.89–0.99 by GraphPad Prism). Detailed IC_{50} values are presented in Table 1.

Table 1. Antioxidant activity of selected medicinal plant extracts — Bergenia, Santolina, Artemisia — by DPPH assay

Sample Code	Plant Identity	DPPH IC_{50} (µg/ml, Mean ± SEM)
Ascorbic acid	Reference standard	31.03 ± 0.016
BEG-4	Bergenia sp.	33.25 ± 0.058
ART-2	Artemisia sp.	837.3 ± 0.096
ST-5	Santolina sp.	993.2 ± 0.035

Lower IC_{50} values indicate stronger antioxidant activity. Samples ranked by ascending IC_{50}. Data expressed as mean ± SEM (n = 4).

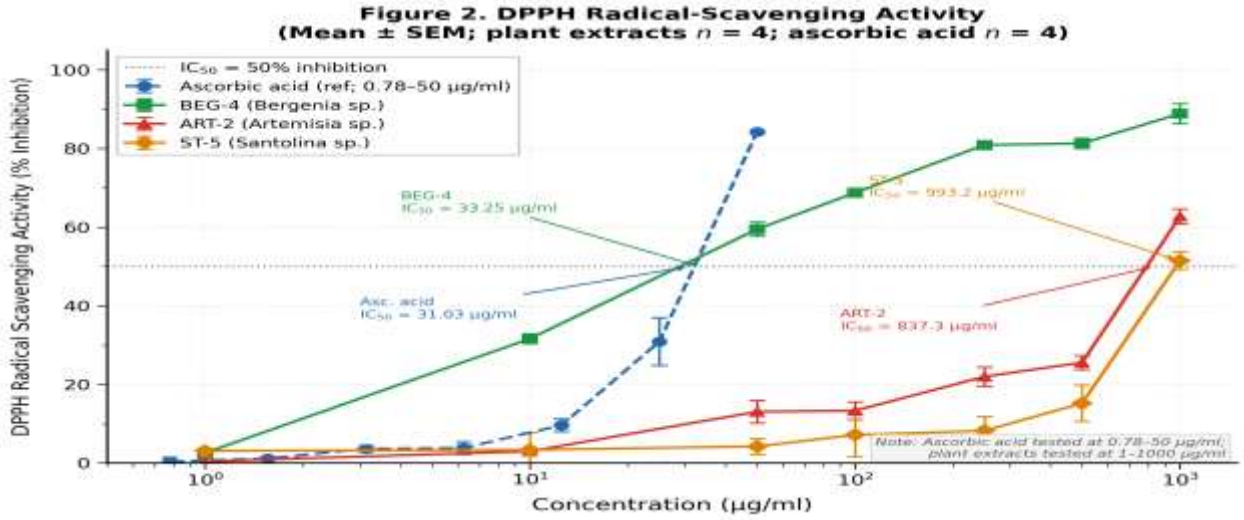

Figure 2. Dose–response curves for the DPPH radical-scavenging assay for Artemisia sp. (ART-2), Bergenia sp. (BEG-4), and Santolina sp. (ST-5), relative to ascorbic acid. Percentage inhibition (mean ± SEM, n = 4) is plotted against extract concentration (µg/ml).

Antibacterial Activity — Disc Diffusion, E. coli (Bergenia, Santolina, Artemisia)
All three plant extracts demonstrated antibacterial activity against Escherichia coli (MTCC-452) by the Kirby-Bauer disc diffusion method. The positive control ciprofloxacin produced a zone of inhibition of 27 mm at 8 µg/disc, confirming satisfactory assay performance. At the lowest effective concentration of 62.5 µg/disc, BEG-4 and ST-5 each produced average inhibition zones of 6 mm, while ART-2 produced a smaller zone of 2 mm. At higher concentrations, zone sizes increased progressively: BEG-4 reached 8 mm at 1000 µg/disc, ART-2 reached 7 mm, and ST-5 reached 6 mm at 1000 µg/disc. The vehicle control (DMSO alone) produced no inhibition zone, confirming that the solvent did not contribute to activity. All three extracts showed substantially weaker activity than ciprofloxacin, which is expected for crude hydroethanolic extracts. Results are summarized in Table 2.
Table 2. Antibacterial activity of selected medicinal plant extracts — Bergenia, Santolina, Artemisia — against Escherichia coli (Kirby-Bauer disc diffusion method)

Sample ID	62.5 µg/disc	125 µg/disc	250 µg/disc	1000 µg/disc
Ciprofloxacin (PC, 8 µg)	27 mm	—	—	—
ART-2	2 mm	6 mm	6 mm	7 mm
BEG-4	6 mm	7 mm	7 mm	8 mm
ST-5	6 mm	—	—	6 mm

Values represent average zone of inhibition (mm). PC = positive control. — = not separately reported at that dose for this extract. All three extracts showed no zone with DMSO vehicle control.

Cytotoxic Activity Against MCF-7 Cells — MTT Assay (Bergenia, Santolina, Artemisia)

The MTT assay demonstrated variable cytotoxic effects of the three plant extracts on MCF-7 breast cancer cells after 24 h exposure. ST-5 (Santolina sp.) showed the strongest cytotoxic activity with an IC_{50} of 54.03 ± 0.110 µg/ml. ART-2 (Artemisia sp.) followed closely with an IC_{50} of 66.56 ± 0.091 µg/ml. BEG-4 (Bergenia sp.) displayed substantially weaker cytotoxicity with an IC_{50} of 964.2 ± 0.053 µg/ml. Dose–response curves for each extract showed progressive reduction in cell viability with increasing concentration, confirming the concentration-dependent nature of the cytotoxic effect. The positive control paclitaxel produced an IC_{50} of 53.65 ± 0.173 nM, confirming assay validity. It is important to note that paclitaxel IC_{50} is expressed in nM while plant extract IC_{50} values are in µg/ml; these units are not directly comparable and no potency equivalence between paclitaxel and the plant extracts should be inferred from the numerical values. IC_{50} values for all extracts are presented in Table 3.

Table 3. Cytotoxic activity of selected medicinal plant extracts — Bergenia, Santolina, Artemisia — against MCF-7 cells (MTT assay)

Sample Code	Plant Identity	IC_{50} (Mean ± SEM)	Unit
Paclitaxel (PC)	Positive control	53.65 ± 0.173	nM*
ST-5	Santolina sp.	54.03 ± 0.110	µg/ml
ART-2	Artemisia sp.	66.56 ± 0.091	µg/ml
BEG-4	Bergenia sp.	964.2 ± 0.053	µg/ml

*Paclitaxel IC_{50} is expressed in nM; plant extract IC_{50} values are in µg/ml. Units are not directly comparable. PC = positive control. Data expressed as mean ± SEM (n = 3).

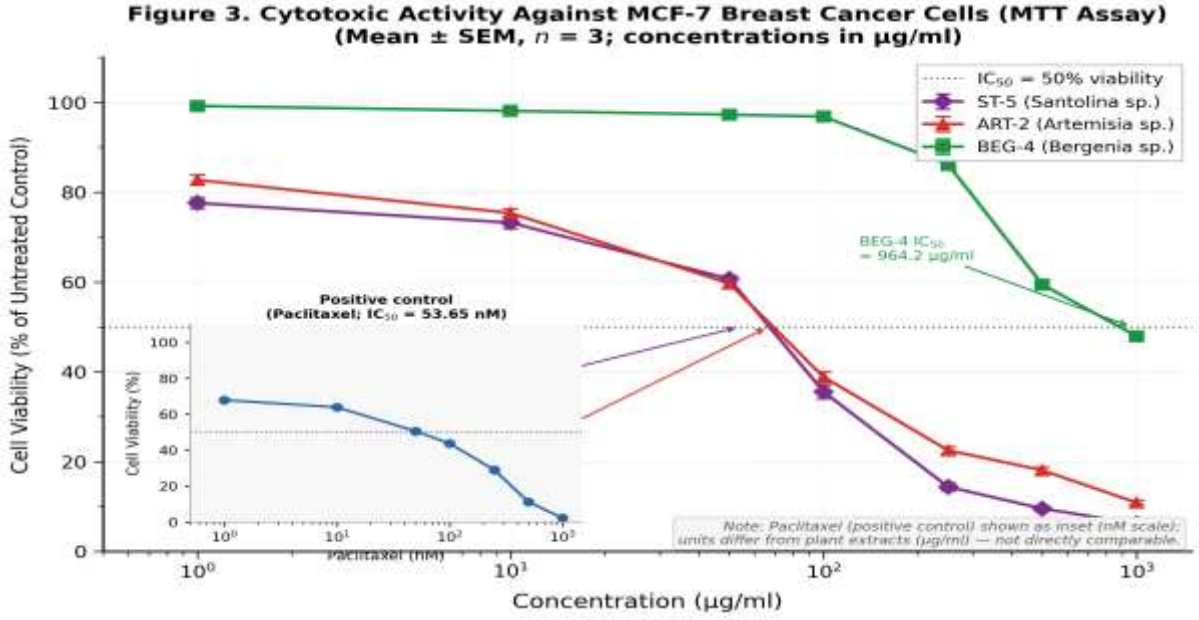

Figure 3. Dose–response curves for the MTT cytotoxicity assay in MCF-7 cells for Artemisia sp. (ART-2), Bergenia sp. (BEG-4), and Santolina sp. (ST-5), with paclitaxel as positive control. Percentage viable cells (mean ± SEM, n = 3) is plotted against extract concentration (µg/ml).

Relative Potency Ranking (Bergenia, Santolina, Artemisia)

On the basis of IC_{50} values, the cytotoxic potency order was: ST-5 > ART-2 >> BEG-4. The antioxidant potency order was the inverse of the cytotoxic ranking for the top and bottom performers: BEG-4 >> ART-2 > ST-5. The antibacterial potency order was: BEG-4 = ST-5 > ART-2.

DISCUSSION

The present study evaluated three Kashmiri medicinal plant extracts — Artemisia sp. (ART-2), Bergenia sp. (BEG-4), and Santolina sp. (ST-5) — in a multi-assay biological screening platform encompassing DPPH antioxidant activity, MCF-7 cytotoxicity, and E. coli antibacterial activity. The results reveal distinct and in several cases divergent bioactivity profiles across the three extracts, with important implications for prioritizing candidates for further phytochemical investigation.

Comparative Cytotoxic, Antioxidant and Antimicrobial Potential of Artemisia

ART-2 (Artemisia sp.) exhibited the second strongest cytotoxic activity among the three extracts, with an IC_{50} of 66.56 ± 0.091 µg/ml against MCF-7 cells. This finding is consistent with the well-established cytotoxic pharmacology of the Artemisia genus, which is rich in sesquiterpene lactones, particularly artemisinins and their semi-synthetic derivatives. Artemisinins have been shown to induce apoptosis, cell-cycle arrest at G2/M phase, and ROS-mediated mitochondrial dysfunction in breast cancer cell lines [13–16]. The IC_{50} of 66.56 µg/ml for the crude ART-2 extract is in the range reported for crude Artemisia absinthium and related species against MCF-7 and other breast cancer lines [17]. In the DPPH assay, ART-2 showed weaker antioxidant activity (IC_{50} = 837.3 ± 0.096 µg/ml), suggesting that the cytotoxic compounds in this extract — likely sesquiterpene lactones and terpenoids — do not substantially contribute to free-radical scavenging in cell-free conditions. This dissociation between cytotoxic and antioxidant profiles is mechanistically coherent: artemisinins exert cytotoxicity partly through pro-oxidant mechanisms (intracellular ROS generation) rather than antioxidant pathways [13,14]. In the antibacterial assay, ART-2 produced a modest inhibition zone of 2 mm at 62.5 µg/disc against E. coli, which is substantially below ciprofloxacin (27 mm at 8 µg), though zone size increased to 7 mm at 1000 µg/disc. This modest antibacterial activity is consistent with published data for crude Artemisia hydroethanolic extracts against Gram-negative organisms, which are generally less susceptible than Gram-positive bacteria due to their outer membrane barrier [14,15]. Overall, ART-2 is a strong candidate for anticancer fractionation studies.

Comparative Cytotoxic, Antioxidant and Antimicrobial Potential of Bergenia

BEG-4 (Bergenia sp.) presented the most striking dissociation between antioxidant and cytotoxic profiles of the three extracts. It was the most potent antioxidant, with an IC_{50} of 33.25 ± 0.058 µg/ml in the DPPH assay, comparable to the reference standard ascorbic acid (31.03 ± 0.016 µg/ml), yet it was the weakest cytotoxic extract with an IC_{50} of 964.2 ± 0.053 µg/ml in the MTT assay. This divergence is consistent with the well-established limitation of cell-free antioxidant assays as predictors of cellular bioactivity [20,21]. It may be explained by several non-exclusive mechanisms. First, the dominant antioxidant phytochemicals in Bergenia — notably bergenin, gallic acid, catechin, and condensed tannins [10,11] — are potent free-radical quenchers in solution but may not efficiently penetrate the MCF-7 cell membrane or accumulate at intracellular concentrations sufficient for cytotoxicity. Second, the cytoprotective nature of bergenin and gallic acid, which can neutralize endogenous ROS required for cancer cell death, may paradoxically reduce rather than enhance antiproliferative activity in the MTT assay context [10,11]. Third, the crude extract may dilute both activity profiles, and bioassay-guided fractionation separating polyphenolic antioxidant fractions from other phytochemical classes may uncover compartmentalized cytotoxic activity. Despite its weak MTT performance, BEG-4 should not be dismissed: its near-reference antioxidant potency has strong relevance for oxidative stress-related disease prevention, and published reports on Bergenia constituents demonstrate anticancer activity for purified bergenin and galloylated compounds in isolated compound studies [10,11]. In the antibacterial assay, BEG-4 produced a zone of 6 mm at 62.5 µg/disc against E. coli, increasing to 8 mm at 1000 µg/disc, making it the most active antibacterial extract among the three, consistent with the well-documented antibacterial properties of gallic acid and tannins against Gram-negative organisms.

Comparative Cytotoxic, Antioxidant and Antimicrobial Potential of Santolina

ST-5 (Santolina sp.) exhibited the strongest cytotoxic activity among the three extracts, with an IC_{50} of 54.03 ± 0.110 µg/ml against MCF-7 cells, ranking it as the most promising candidate for anticancer fractionation. Santolina species are phytochemically characterized by monoterpenes (notably camphor, α-pinene, and 1,8-cineole in the essential oil), sesquiterpene lactones, flavonoids (including luteolin and apigenin derivatives), and coumarins, all of which have been individually reported to possess antiproliferative activity [22,23]. The relatively low IC_{50} of ST-5 is likely attributable to the combined cytotoxic contributions of these phytochemical classes. The possible mechanisms include induction of apoptosis via mitochondrial pathway activation, inhibition of pro-survival kinases, and disruption of microtubule dynamics — pathways well-documented for flavonoids and monoterpenes in breast cancer models [22–35]. In the DPPH assay, ST-5 was the weakest antioxidant (IC_{50} = 993.2 ± 0.035 µg/ml), indicating that the cytotoxic compounds in this extract do not substantially contribute to cell-free radical scavenging, which further supports the hypothesis that terpenoid and flavonoid aglycone fractions drive cytotoxicity through mechanisms other than simple antioxidant activity. In the antibacterial assay, ST-5 produced a zone of 6 mm at 62.5 µg/disc against E. coli, consistent with the reported moderate antibacterial

activity of Santolina essential oils and extracts against Gram-negative bacteria. Together, these data establish ST-5 as the highest-priority candidate for bioassay-guided fractionation and mechanistic anticancer investigation in this extract panel.

Regarding the antibacterial data collectively, all three extracts showed measurable activity against E. coli, with zones substantially smaller than ciprofloxacin (27 mm at 8 µg/disc), which is consistent with the generally modest antibacterial potency of crude hydroethanolic plant extracts in disc diffusion systems. These preliminary findings are hypothesis-generating and should be followed by MIC determination and evaluation against a broader panel of Gram-positive and Gram-negative organisms to establish the antibacterial spectrum.

The present data set has important limitations. The study used crude extracts and a single cancer cell line (MCF-7). Cytotoxicity against a normal mammary epithelial cell line (e.g., MCF-10A) was not assessed, so selectivity index (SI = IC_{50} normal / IC_{50} cancer) cannot be calculated. A high selectivity index is a critical criterion for identifying candidate extracts for further development, and its absence limits the translational value of the current findings. Future studies should determine SI values, employ additional cancer cell lines, perform apoptosis and cell-cycle analysis, and conduct bioassay-guided fractionation to identify the active phytochemicals. Total phenolic and flavonoid contents should also be quantified to establish structure-activity correlations. Extraction yield and species-level botanical identification for ART-2, BEG-4, and ST-5 should also be reported.

CONCLUSION

Three medicinal plant extracts obtained from the Floriculture Development Scheme, Lal Mandi, Srinagar — Artemisia sp. (ART-2), Bergenia sp. (BEG-4), and Santolina sp. (ST-5) — were evaluated in a multi-assay biological screening platform. The extracts exhibited distinct and partially divergent bioactivity profiles. ST-5 showed the strongest cytotoxic activity (IC_{50} = 54.03 ± 0.110 µg/ml against MCF-7 cells), followed closely by ART-2 (IC_{50} = 66.56 ± 0.091 µg/ml). BEG-4 was the most potent antioxidant (IC_{50} = 33.25 ± 0.058 µg/ml in the DPPH assay), comparable to ascorbic acid, while ART-2 and ST-5 were substantially weaker antioxidants. In the antibacterial assay against E. coli, BEG-4 and ST-5 produced 6 mm inhibition zones and ART-2 produced 2 mm at 62.5 µg/disc. ST-5 and ART-2 are identified as priority candidates for phytochemical fractionation and mechanistic anticancer investigation, while BEG-4 warrants further study of its antioxidant and antibacterial constituents and their potential antiproliferative activity upon isolation.

Limitations

This study was conducted using crude hydroethanolic extracts and a single cancer cell line (MCF-7). The data set would be substantially strengthened by: (1) testing on normal mammary epithelial cells (e.g., MCF-10A) to determine selectivity index; (2) evaluation against multiple cancer cell lines; (3) apoptosis markers and signaling pathway analysis; (4) bioassay-guided fractionation and isolation of active constituents; (5) determination of total phenolic and flavonoid contents to support structure–activity interpretation; (6) accurate reporting of extraction yield (%w/w) for reproducibility; (7) species-level botanical identification and herbarium voucher deposition; and (8) MIC-based antibacterial testing against a broader panel of microorganisms.

Declarations

Ethics Approval and Consent to Participate

Not applicable for in vitro cell-line and disc diffusion screening. Plant collection was conducted under the source records of the Floriculture Development Scheme, Lal Mandi, Srinagar, Jammu and Kashmir, India.

Consent for Publication

Not applicable.

Availability of Data and Materials

All raw data, including antioxidant (DPPH) data files, MTT assay data, and antibacterial disc diffusion data, are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no competing interests.

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No external funding was reported for this screening study.

Authors' Contributions

Conceptualization: **S.F :** Writing – original draft. **S.F,A.H.S :** Methodology: **S.F :**Writing – review & editing :**S.F, A.H.S, W.U.R, S.A.S ..** All authors reviewed and approved the final manuscript version.

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Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; MCF-7, Michigan Cancer Foundation-7; MHA, Mueller-Hinton Agar; MIC, minimum inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTCC, Microbial Type Culture Collection; NCCS, National Centre for Cell Science; ROS, reactive oxygen species; SEM, standard error of mean; SI, selectivity index; TFC, total flavonoid content; TPC, total phenolic content.

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