Original Article

Studies on Phytoconstituents, *in vitro* Antioxidant, Antibacterial, Antiparasitic, Antimicrobial, and Anticancer Potential of Medicinal Plant *Lasiosiphon eriocephalus* Decne (Family: *Thymelaeaceae*)

Pratik P. Durgawale, Madhavi N. Patil, Shreepad A. Joshi, Kalpita S. Korabu, Kailas D. Datkhile

Department of Molecular Biology and Genetics, Molecular and Genetic Laboratory, Krishna Institute of Medical Sciences University, Satara, Maharashtra, India

Abstract

Background: The present study has been undertaken with an objective to determine the antioxidant, antimicrobial, antiparasitic, and cytotoxic activity of endangered medicinal plant *Lasiosiphon eriocephalus.* **Materials and Methods:** Aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers of the selected medicinal plant were screened for the presence of phenolics, tannins, and flavonoids. *In vitro* antioxidant activity was evaluated by 1,1-diphenyl-2-picryl hydrazine and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) assay. The antiparasitic activity of extracts was tested against protozoan endoparasite *Leishmania donovani*, and antimicrobial activity plant extracts were determined against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The anticancer properties of plant extracts were studied by cytotoxicity on cancer cells including HeLa and MCF-7 determined by MTT assay and DNA fragmentation assay. **Results:** The extracts screened for phytochemical analysis was found to contain phenolics, tannins, and flavonoids. The water and solvent extracts of leaves, and flowers had shown strong antioxidant activity in vitro. The leaves and flower extract showed the ability to inhibit the growth of both Gram-positive as well as Gram-negative bacteria. The results also showed increased antimicrobial and antiparasitic activities of ethanol extracts of flowers then leaves and followed by bark. The present study revealed that the treatment of crude extract of leaves as well as stem bark of *L. eriocephalus* exhibited strong cytotoxic activity against tested cancer cell lines. **Conclusion:** These results indicated that the possible secondary metabolites from the crude extract of *L. eriocephalous* plant have a potential as antioxidant, antiparasitic, antibacterial, and cytotoxic agent against cancer cells. However, further study needs to be carried out to understand the detailed molecular mechanism involved in the inhibition of cell proliferation and induction of apoptosis in response to phytoconstituents derived from *L. eriocephalus*.

Keywords: Antimicrobial activity, antioxidants, antiparasitic activity, cytotoxicity, *Lasiosiphon eriocephalus*

Introduction

Naturally occurring phytochemicals from medicinal plants contain a wide range of chemical substances with important therapeutic properties which have acquired substantial contribution in the treatment of deadly diseases. According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary health-care needs. The plant kingdom holds many plant species containing substances of medicinal value which have yet to be discovered. Therefore, nowadays, interest has been developed to elucidate the mode of action of number of phytochemicals from different medicinal plants. Several studies have been conducted to reveal their antioxidant,[1,2] antibacterial,[3-5] and antiparasitic[6,7] properties. In recent years, medicinal plants and plant-derived products have also attracted researchers due to their diverse range of therapeutic properties in the management of various cancers.[8-10]

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Address for correspondence: Dr. Kailas D. Datkhile, Department of Molecular Biology and Genetics, Molecular and Genetic Laboratory, Krishna Institute of Medical Sciences (Deemed To Be University), Taluka-Karad, Satara - 415 110, Maharashtra, India.
E-mail: hodgeneticslab@kimsuniversity.in

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Lasiosiphon eriocephalus Decne commonly known as Raamethaa (Family: *Thymelaeaceae*) is widely used in the treatment of many aliments. It has widely distributed all over India, mostly in Maharashtra, Kerala, Karnataka, and throughout Western Ghats. Phytochemical studies on several species of family *Thymelaeaceae* showed the presence variety of different classes of natural products including secondary metabolites such as tannins, glycosides, coumarins, and flavonoids linearly distributed in plant parts such as leaves, stem bark, roots, and fruits.[11-14] There are few reports on phytochemicals and their pharmacological activities in different plant parts of these plants.[15] Earlier, several genera of *Thymelaeaceae* are considered for their biological activities such as anticancer,[16] antileukemic,[17] antidiabetic,[18] antiplasmodial,[19] and cytotoxicity[20] activities. Similarly, phytochemicals from different species of *Thymelaeaceae* have been reported to show antioxidant activity in *Gnidia stenophylla*,[21] antibacterial in (*Gonia capitata*),[22-23] antifungal in *(G. kraussianus),*[24] and antiparasitic in (*G. cuneata)* activities.[19,25] Furthermore, *L. eriocephalus* have tremendous pharmacological potential which had been used traditionally for long time as substitutional medicine for a variety of medicinal complaints in humans including dropsy, sores, burns, constipation, coughs, earache, epilepsy, headache, fevers, insanity, malaria, measles, pulmonary tuberculosis, smallpox, snake bites, sprains and fractures, tonsillitis, stomach and chest complaints, toothache, and ulcers.[26]

Although earlier studies reported curative properties of active phytoconstituents from other genera of family *Thymelaeaceae* including *Daphne, Gnidia, Wikstroemia,* and *Pimelea* species,[16] lesser efforts have been made to elucidate either of antimicrobial, antiparasitic, cytotoxicity, or genotoxicity properties of *L. eriocephalus*. Therefore, in this study, we selected this traditional endangered medicinal plant with an aim investigate the effect of different solvents extractions on phytochemicals and to evaluate antioxidant, antibacterial as well as antiparasitic properties of different solvent extracts of *L. eriocephalus*. This study was further extended to study cytotoxic and genotoxic properties of different solvent extracts of leaves, stem bark, and flowers of *L. eriocephalus.*

Materials and Methods

Chemicals and reagents

2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picryl hydrazine (DPPH) were obtained from Sigma Aldrich. Butylated hydroxytoluene (BHT), deoxyribose, nitroblue tetrazolium, Phenazine methosulfate, ascorbic acid, Potassium persulfate, phosphate buffer, potassium ferricyanide, thiobarbituric acid, trichloroacetic acid, ferric chloride, ethylenediaminetetraacetic acid (EDTA), and the solvents were obtained from Sisco Research Laboratories. Minimum Essential Medium (10370-021), fetal bovine serum (FBS) (2614079), RPMI1640 (11875-085), and penicillin-streptomycin (15140-122) were purchased from Thermo Fisher Scientific, DMSO (D2650), purchased from Sigma Aldrich, MTT (M6494) from Invitrogen, 6 well plates (CLS3355), 96 well plates (CLS3599), and 5 ml serological pipettes (CLS4487) purchased from Corning.

Collection and processing of plant materials

Plant parts were collected from Amboli Ghat region of Maharashtra from India and authenticated by the Department of Botany, University of Pune, India. The plant parts were thoroughly washed with double-distilled water (ddH_2O) for removal of adhered dust particles, shade dried and then ground into fine powder using mortar and pestle. The powdered samples were stored in an air-tight container in the dark at room temperature for further experiments.

Preparation of solvent extracts

Based on the published literature, 25 g of powdered leaf, stem bark, and flowers were used for solvent extraction. Soxhlet extraction was carried out in different solvents, namely distilled water, methanol, and ethanol. The extracts obtained were evaporated in crucible dishes kept in desiccators till dryness. The dried extracts were dissolved in their respective solvents to get known concentration of the extracts (10 mg/mL) and preserved at 4°C in airtight bottles until further use.

Phytochemical screening of *Lasiosiphon eriocephalus* plant extracts of different plant parts

Determination of total polyphenolic content

The total soluble polyphenolic content of plant extracts from different plant parts was determined by the Folin–Ciocalteu reagent method. The reaction mixture containing 100 µL of sample and 750 µL of 7.5% sodium carbonate (Na_2CO_3) solution, 250 µL of Folin–Ciocalteu reagent and the reaction was incubated at 37°C for 2 h (h) with intermittent shaking, and the absorbance was measured at 765 nm. Gallic acid (100 µg/mL) was used for calibration of the standard curve. The results were expressed as microgram of gallic acid equivalent (mg GAE)/g of dry plant material.

Determination of tannin content

Total tannin content of plant extracts from different plant parts was determined by the Folin–Ciocalteu reagent method. The reaction mixture containing 100 µL of (10–100 µg/mL) plant extract in 750 µL of ddH_2O, 50 µL of Folin–Ciocalteu reagent, and 200 µL of 17.5% Na_2CO_3 was mixed thoroughly, and the reaction was incubated at 37°C for 30 min with intermittent shaking and the absorbance was measured at 725 nm using tannic acid as standard. Tannic acid (20–100 µg/mL) was used for calibration of standard curve. The results were expressed as mg of tannic equivalent to Gram of dry plant material.

Estimation of total flavonoid content

The total flavonoid content was determined using the Dowd method. 200 µL of different concentrations of plant extracts in water, methanol, and ethanol were mixed with add 100 µL of a 5% (w/v) NaNO2 and mixed vigorously, After 5 min, 200 µL of 10% (w/v) aluminum trichloride was added in each tube and allowed to stand for a further 10 min at room temperature. 500 µL of 1 M NaOH was added finally and the volume of

mixture was adjusted to 2.0 mL with ddH_2O. The absorbance of reaction was measured at 510 nm against blank samples. The total flavonoid content was determined as microgram of rutin equivalent by using an equation that was obtained from standard graph using standard rutin solution (10–100 µg/mL) prepared in ethanol.

Evaluation of antioxidant properties of *Lasiosiphon eriocephalus* plant extracts

Antioxidant properties were determined by free radical scavenging assays such as DPPH and ABTS assay.

1,1-diphenyl-2-picryl hydrazine radical scavenging assay

The DPPH scavenging activity of aqueous, methanol, and ethanol extracts of different plant parts was measured according to the method described by RajanRushender *et al*., 2012[27] with some modifications. The reaction mixture consisted of a mixture of freshly prepared 1 mL DPPH (0.1 mM) solution in methanol with different concentrations (25, 50, 100, 200, and 400 µg/mL of aqueous, methanol, and ethanol extracts in final volume of 0.1 mL in distilled water or methanol, respectively. The blank sample consisted of 2 mL of methanol, while the control contained 2 mL DPPH methanolic solution only. The absorbance of sample, blank, and control was measured at 517 nm, after 30 min incubation of reaction in the dark at room temperature, using an ultraviolet (UV)-Visible 1800 spectrophotometer (Shimadzu). The percentage of DPPH scavenging activity was calculated using the expression below: Percentage inhibition (%) = (A0–A1)/A0) ×100, where: A0 is the Absorbance of control and A1 Absorbance of test. The results were compared with ascorbic acid and BHT as standard.

2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay

ABTS radical scavenging assay was carried out using procedures described by Arnao *et al*. 2001.[28] ABTS•+ radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.4 mM) and incubating the mixture at room temperature in the dark for 16 h. Different concentrations of aqueous as well as methanolic extracts in final volume of 0.1 mL were added and allowed to react with 1 mL of ABTS for 30 min and the absorbance was recorded at 734 nm after incubation. The blank sample consisted of 1 mL of methanol, while the control contained 1 mL ABTS solution prepared in methanol. Ascorbic acid and BHT were used as standard. The percentage of inhibition was calculated from the following equation:

Percentage inhibition (%) = (A0–A1)/A0) ×100

Evaluation of antimicrobial properties of *Lasiosiphon eriocephalus* plant extracts

The antimicrobial activity of plant extracts was performed against Gram-positive Bacteria *Staphylococcus aureus* (ATCC® 29213™) and Gram-negative bacteria, *Escherichia coli* (ATCC® 25922™), *Klebsiella pneumoniae* (ATCC® 700603™) and *Pseudomonas aeruginosa* (ATCC® 2617™). In a sterile, 1.5 mL microcentrifuge tube, the sample solutions at different concentrations were tested on bacterial solutions in 1 mL of Muller Hinton culture medium incubated at 37°C for 24 h. Ampicillin instead of the sample solution, sterile water instead of bacterial solution as positive control and either methanol or ethanol solvents were used as negative controls for each assay. After incubation period, bacterial growth inhibition (GI) was evaluated by spectrophotometer by checking absorbance at 600 nm. The activity of the sample was calculated as percentage of bacterial % GI according to the following equation: % GI = (Negative correction – Sample correction)/Negative correction × 100. Where, Negative correction: Absorbance of negative control–Absorbance of sterility control; Sample correction: Absorbance of the samples–Absorbance of color control.

Evaluation of antiparasitic properties of *Lasiosiphon eriocephalus* plant extracts

Leishmania donovani parasites used in the assays were transformed from promastigote to amastigote forms transferring 1 mL of promatigotes log phase culture into 5 mL of RPMI1640 supplemented with 10% heat-inactivated FBS and maintained at 25°C until its use in bioassays. Primary screening of different plant extracts was performed by exposure of *L. donovani* cells at different concentrations of extracts for 48 h. The inhibition of parasites growth in culture was determined by observing under phase contrast microscope for their morphology.

Evaluation of anticancer properties of *Lasiosiphon eriocephalus* plant extracts

In vitro evaluation of cytotoxicity properties of Lasiosiphon eriocephalus plant extract

In vitro cytotoxicity effects of *L. eriocephalus* leaves, stem bark and flower extracts the on human cancer cell lines including HeLa and MCF-7 cells were determined by the MTT assay. The cells were maintained in MEM medium supplemented with 10% FBS, Penicillin-Streptomycin at 100 U/mL, 100 µg/mL in a humidified atmosphere of 5% CO_2 at 37°C. In a 96 well plate, 10,000 cells were added in 100 µL of MEM medium and incubated at 37°C, 5% CO_2. After 24 h incubation, the confluent cells were exposed to respective treatment of extracts at concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL in culture medium without FBS and incubated for further 48 h at 37°C and 5% CO_2. After 48 h completion of treatment, cells were washed with sterile Hanks' Balanced Salt Solution thereafter 10 µL per well (10 µL/well) of MTT (5 mg/ml) added to the cells and the plates were further incubated 37°C, 5% CO_2 atmosphere. After 4 h incubation, the MTT solution was carefully removed and 200 µL of DMSO was added to each well. The absorbance of purple color developed was measured at 570 nm wavelength using UV-Vis 1800 spectrophotometer (Shimadzu). The results were expressed as percentage of cell survival as compared to the control. All experiments were performed in triplicates. The morphology of the cells was then examined in an inverted phase-contrast microscope (Primovert Carl Zeiss microscope).

DNA fragmentation assay

The apoptotic activity of the extracts of *L. eriocephalus* was determined by DNA fragmentation assay. HeLa and MCF-7 cells (1×10^6) in 6 well plates were treated with 50% inhibitory concentration (IC_{50}) and 90% inhibitory concentration (IC_{90}) concentration of extracts along with untreated control cells, cells treated with solvents used for preparation of extracts and incubated at 37°C in 5% CO_2, for 48 h. Cells were harvested, followed by lysis in 0.3 mL of cell lysis buffer containing (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.2% Triton X-100, and 0.5% sodium dodecyl sulfate). After treatment with 0.5 mg/mL of Ribonuclease A (RNase-A) at 37°C for 1 h, thereafter, 0.2 mg/mL of Proteinase-K at 55°C for 1 h, DNA was precipitated by addition of equal volume of isopropanol and 1/10th volume of 5 Molar (M) sodium chloride. The DNA pellet was resuspended in Tris-EDTA (TE) buffer and DNA was separated on 1.5% (w/v) low-EEO agarose (GeNei) gel containing 1 μg/mL ethidium bromide and subjected to electrophoresis at 80 volts (V) for 1–2 h in Tris-Acetate-EDTA buffer along with 100 base pair (bp) and 1 kilo bp DNA molecular weight marker. The DNA fragments were visualized by exposing the gels to UV transilluminator followed by photography in gel documentation system (BioRad Laboratories, USA).

Statistical analysis

All experiments were done in three replicates and then values were expressed as mean ± standard error of three measurements. One-way analysis of variance and Student's *t*-test were carried out using Microsoft Excel 2010 to evaluate the possible differences among the means. There were significant differences with $P \leq 0.05$.

Results

Phytochemical analysis

Preliminary phytochemical screening of the aqueous, methanol and ethanol extracts of different plant parts including leaves, stem bark, and flowers of *L. eriocephalus* revealed the presence of various groups of compounds (phenolics, tannins, and flavonoids). These data were obtained according to the colorimetric analyses quantitated using standard curve of gallic acid, tannic acid, and rutin as standard solutions. The results are summarized in Figure 1.

In vitro antioxidant activity

The *in vitro* DPPH and ABTS radical scavenging activity of different solvent extracts of *L. eriocephalus* is shown in Figure 2a and b. The highest DPPH radical scavenging activity was observed in the methanol and ethanol extracts of flowers, while aqueous extract also showed comparatively lower effect. The results showed that the aqueous extract of leaves exhibited comparatively lower DPPH radical scavenging ability (IC_{50}:184.07 ± 23.77 μg/mL) than the methanol (IC_{50}: 131.35 ± 15.68 μg/mL) and ethanol extracts (IC_{50}: 46.95 ± 3.28 μg/mL). IC_{50} value of aqueous extract of stem bark and flowers were observed as

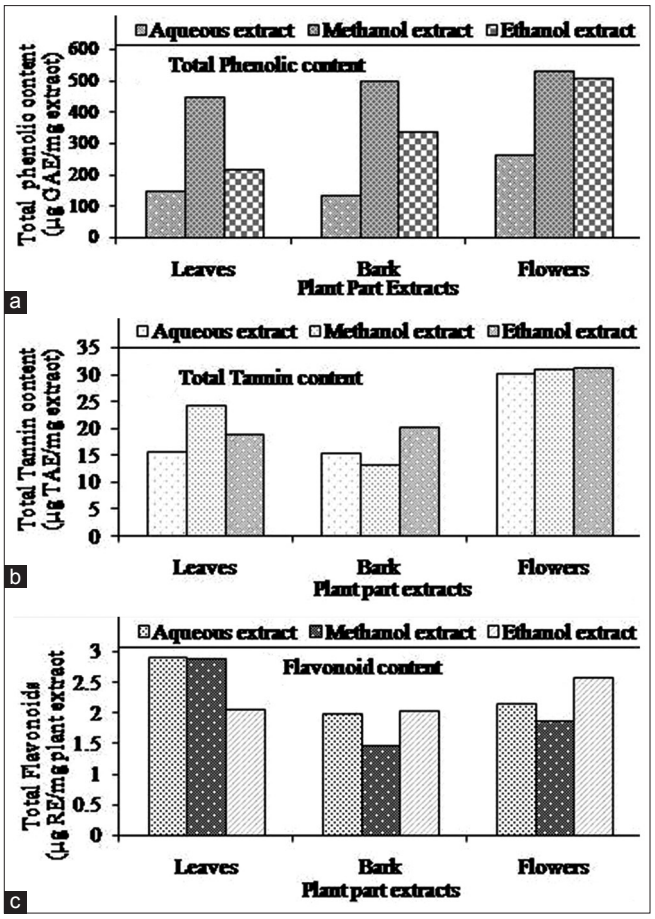

Figure 1: Representative histogram showing (a) total phenolic content (b) total tannin content and (c) flavonoid content in the aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers of *Lasiosiphon eriocephalus*. Values are expressed as the means of three independent experiments

(118.11 ± 11.93 μg/mL) and (52.22 ± 7.02 μg/mL) and methanol extract (77.34 ± 10.97 μg/mL; 51.81 ± 5.73 μg/mL and ethanol extracts (59.70 ± 0.54 μg/mL) and (44.57 ± 0.51 μg/mL), respectively. Thus, DPPH radical scavenging activity of ethanol extracts of flowers was significantly higher than aqueous and methanol extracts of both stem bark and leaves. Similarly, when we checked ABTS radical scavenging activity of extracts of *L. eriocephalus* plant parts, aqueous, methanol, and ethanol extracts of flowers showed significantly increased radical scavenging activity than stem bark and leaves. IC_{50} values of aqueous extract of leaves, stem bark, and flowers were 62.24 ± 1.10 μg/mL; 61.17 ± 0.53 μg/mL; and 42.78 ± 0.77 μg/mL, methanol extract was 25.36 ± 0.42 μg/mL; 30.88 ± 1.55 μg/mL; 23.38±0.74 μg/mL, and ethanol extracts were 21.90±0.72 μg/mL; 19.95 ± 1.05 μg/mL; and 15.06 ± 0.25 μg/mL, respectively.

Antimicrobial activity of tested extracts of *Lasiosiphon eriocephalus*

The antibacterial activities of the aqueous, methanol, and ethanol extracts were assayed *in vitro* by minimum inhibitory concentration method against four bacteria species, *S. aureus,*

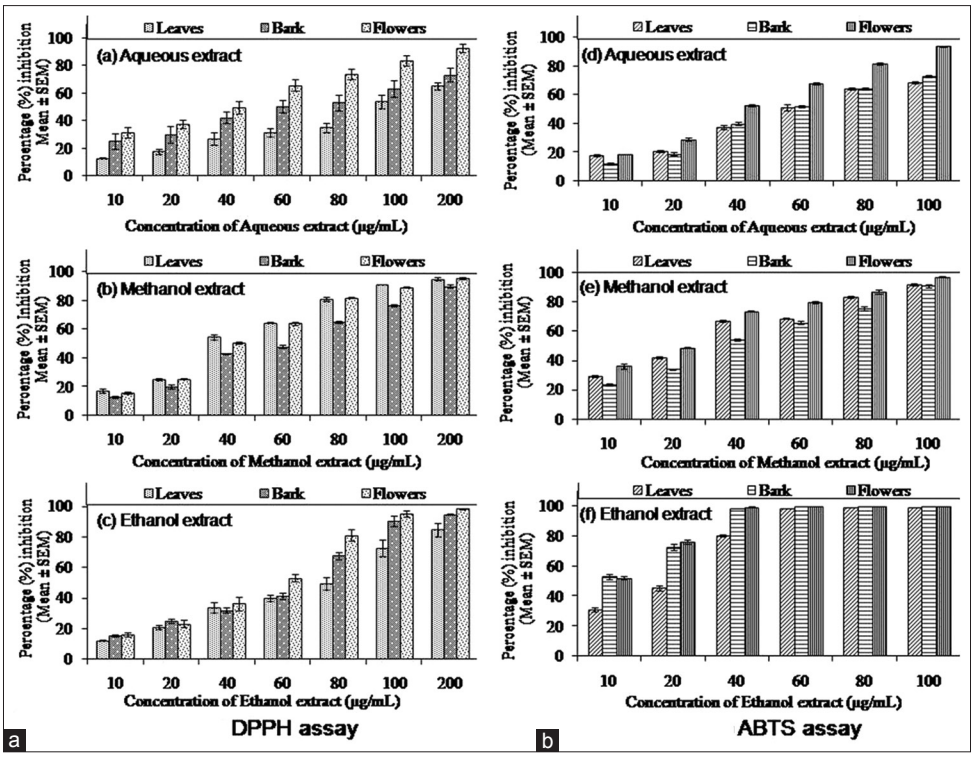

Figure 2: Representative histogram showing (a) 1,1-diphenyl-2-picryl hydrazine and (b) 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of different concentrations of aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers of *Lasiosiphon eriocephalus.* The data represent the percentage inhibition of radicals *in vitro*. The results represent the means of three independent experiments, and error bars represent the standard error of the mean

K. pneumoniae, E. coli, *and P. aeruginosa*. Among the tested solvent extracts, ethanol extract of flowers showed minimum antimicrobial activity in selected bacterial species except *K. pneumoniae* where aqueous extract of leaves showed 18.99% ± 0.21% of inhibition of bacterial cell growth, whereas methanol and ethanol extracts showed 37.54% ±6.63% and 69.78% ±8.96% inhibition of cell growth at concentration of 1 mg/mL [Figure 3c]. Whereas, aqueous, methanol, and ethanol extracts of stem bark of *L. eriocephalus* showed 40.66 ± 4.71, 44.36 ± 7.14, and 71.34 ± 0.35 and flowers showed 39.40 ± 0.39, 57.67 ± 5.49, and 75.57 ± 3.70 inhibition *K. pneaumoniae* cell growth, respectively. The aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers showed the highest inhibition of *E. coli* bacterial cell growth at 1.0 mg/mL concentration [Figure 3a]. Similarly, doses of both aqueous, methanol, and ethanol extracts of leaves stem bark and flowers required to kill *S. aureus, and P. aeruginosa* are lower which showed more than 90% of GI at 1 mg/mL concentration of both leaves, stem bark, and flower extracts[Figure 3b and d].

Antiparasitic activity of tested extracts of *Lasiosiphon eriocephalus*

In the antiparasitic assays, different solvent extracts including water, methanol, and ethanol extracts of leaves [Figure 4a], stem bark [Figure 4b], and flower [Figure 4c] were tested against *L. donovani*. Among all the extracts tested, methanol and ethanol extract of bark and flowers showed the highest antiparasitic activity at the dose of 0.6 mg/mL and 0.4 mg/mL, respectively.

Anticancer activity of tested extracts of *Lasiosiphon eriocephalus*

In order to screen cytotoxicity of aqueous as well as ethanol and methanol extracts of leaves, stem bark and flowers of *L. eriocephalus,* the cell viability was determined by MTT assay. The cytotoxic activity of *L, eriocephalus* extracts on MCF7 cells represented in figure [Figure 5a and b] whereas the activity of extracts on HeLa cells shown in Figure 5c and d. When plant extract was tested for cytotoxic activity, the data obtained from cell viability results of HeLa and MCF-7 cells revealed that methanol extract of leaves and stem bark exhibited a range of significant cytotoxic activities in a dose-dependent manner varying from 0.025–0.6 mg/mL whereas aqueous extract of leaves and stem bark showed decreased cell viability with an increase in the concentration of the extract from 0.025–0.8 mg/mL. The results indicated that when MCF-7 cells were treated with 0.4 mg/mL concentration of methanol and ethanol extract of leaves, stem bark and flowers, the cells showed 100% mortality whereas 0.8 mg/mL concentration of aqueous extract was needed to kill MCF-7 cells. HeLa cells required 0.6 mg/ml of methanol as well as ethanol extract for 100% inhibition. Similarly, 0.8 mg/ml of aqueous extracts of leaves, stem bark and flowers was required to kill MCF-7 and HeLa cells. Microscopic observations of aqueous, methanol as well as ethanol extract of leaves, stem bark, and flowers

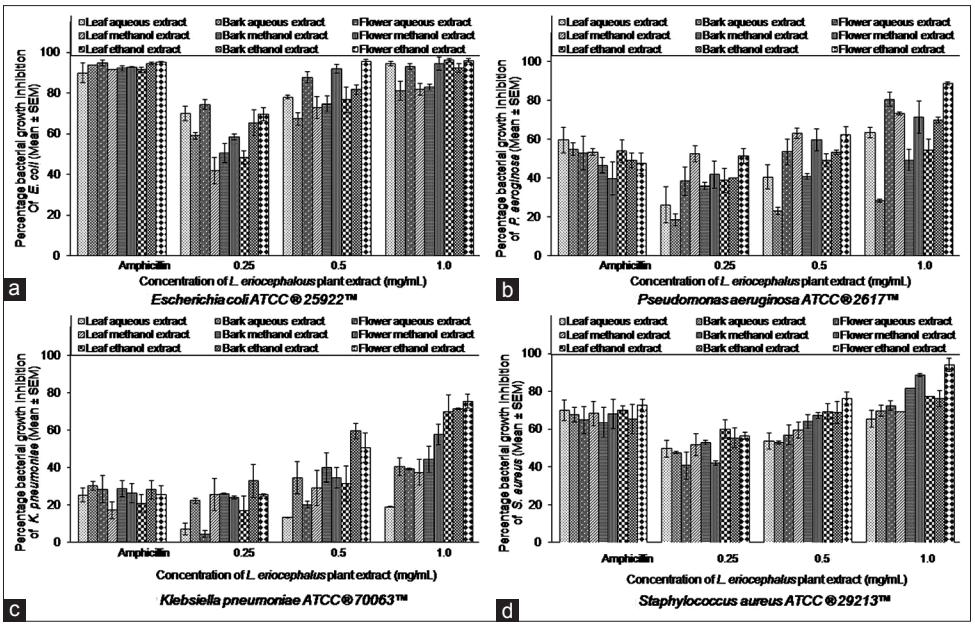

Figure 3: Representative histogram showing bacterial growth inhibition of (a) *Escherichia coli* ATCC® 25922™ (b) *Pseudomonas aeruginosa* ATCC® 2617™ (c) *Klebsiella pneumoniae* ATCC® 70063™ and (d) *Staphylococcus aureus* ATCC® 29213™ exposed to different concentrations (0.2, 0.5, and 1.0 mg/mL) of aqueous, methanol, and ethanol extracts of leaves, stem bark and flowers of *Lasiosiphon eriocephalus* for 24 h. The results represent the means of three independent experiments, and error bars represent the standard error of the mean

treated MCF-7 and HeLa cells showed distinct cellular morphological alterations such as loss of membrane integrity therefore appeared shrunken, cytoplasmic condensation as indicating unhealthy cells, whereas the control cells appeared normal [Figure 5b and d, respectively]. The evaluation of genotoxic effects of IC_{50} and IC_{90} concentrations of methanol as well as aqueous extracts of both leaves and stem bark of *L. eriocephalus* on cancer cells including HeLa and MCF-7 cells were assessed by DNA fragmentation pattern on agarose gel. The methanol and aqueous extract of leaves and stem bark was significantly genotoxic to MCF-7 and HeLa cells as noted by extensive double-strand breaks thereby yielding a ladder appearance when treated with IC_{50} and IC_{90}as concentration of extracts as shown in [Lanes 3 and 4, Figure 6] while the DNA of control cells exhibited minimum or no DNA breakage [Lane 2, Figure 6] of MCF-7 and HeLa cells.

Discussion

In the present study, phytochemical screening, *in vitro* antioxidant, antimicrobial, and cytotoxic activities of different solvent extracts from the *L. eriocephalus* were examined. Phytochemical evaluation was performed with crude aqueous, methanol and ethanol extracts of leaves, stem bark and flowers of *L. eriocephalus* where both methanol and ethanol extracts showed the presence of higher amounts of phytochemicals including phenolics, tannins, and flavonoids. Both methanol and ethanol extracts of flowers were found to be rich in phenols and tannins than aqueous extract. The tested extracts have a strong antioxidant activity against various oxidative systems *in vitro* including DPPH and ABTS radicals. We found that the tested extracts exhibited the higher radical scavenging activity with the greatest amount of phytoconstituents content. Extensive research has been carried out on the free radical scavenging properties of phytochemicals of different parts of various medicinal plants.[29-30] However, the components responsible for the antioxidative activity of *L. eriocephalus* extracts are currently unclear. Earlier, only a single report available on antioxidant properties of *Gnidia glauca* from the family *Thymelaeaceae* stating the antioxidant capacity in stem bark and leaves,[31] but in contrary to that report our results showed marginal increase in antioxidant capacity in the flowers. The phytochemical analysis of *L. eriocephalus* already revealed the presence of secondary metabolites including phenolic, tannins, and flavonoid compounds in this plant extracts could be contributed to its antioxidant properties. Thus, the *in vitro* antioxidant activity of *L. eriocephalus* indicates that the plant extracts may contain compounds that are capable of donating hydrogen to a free radical to remove electrons responsible for the radical's reactivity.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.[3,4] In the present study, among all the tested samples of *L. eriocephalus*, both ethanol and methanol extract of leaves, bark, and flowers showed bacterial growth inhibitory effects against bacterial strains except *K. pneumoniae*. Earlier, roots, seeds, or bark extracts of *L. eriocephalus* showed antibacterial activity, but the higher concentrations of plant extracts are needed to inhibit bacterial cell growth.[32] In contrast to those, our results showed that the lower concentrations of leaf and flowers showed more bacterial GI against *E. coli* and *P. aeruginosa*. The antimicrobial assay revealed that ethanol extract of flowers and leaves of *L. eriocephalus* exhibited broad-spectrum activity against

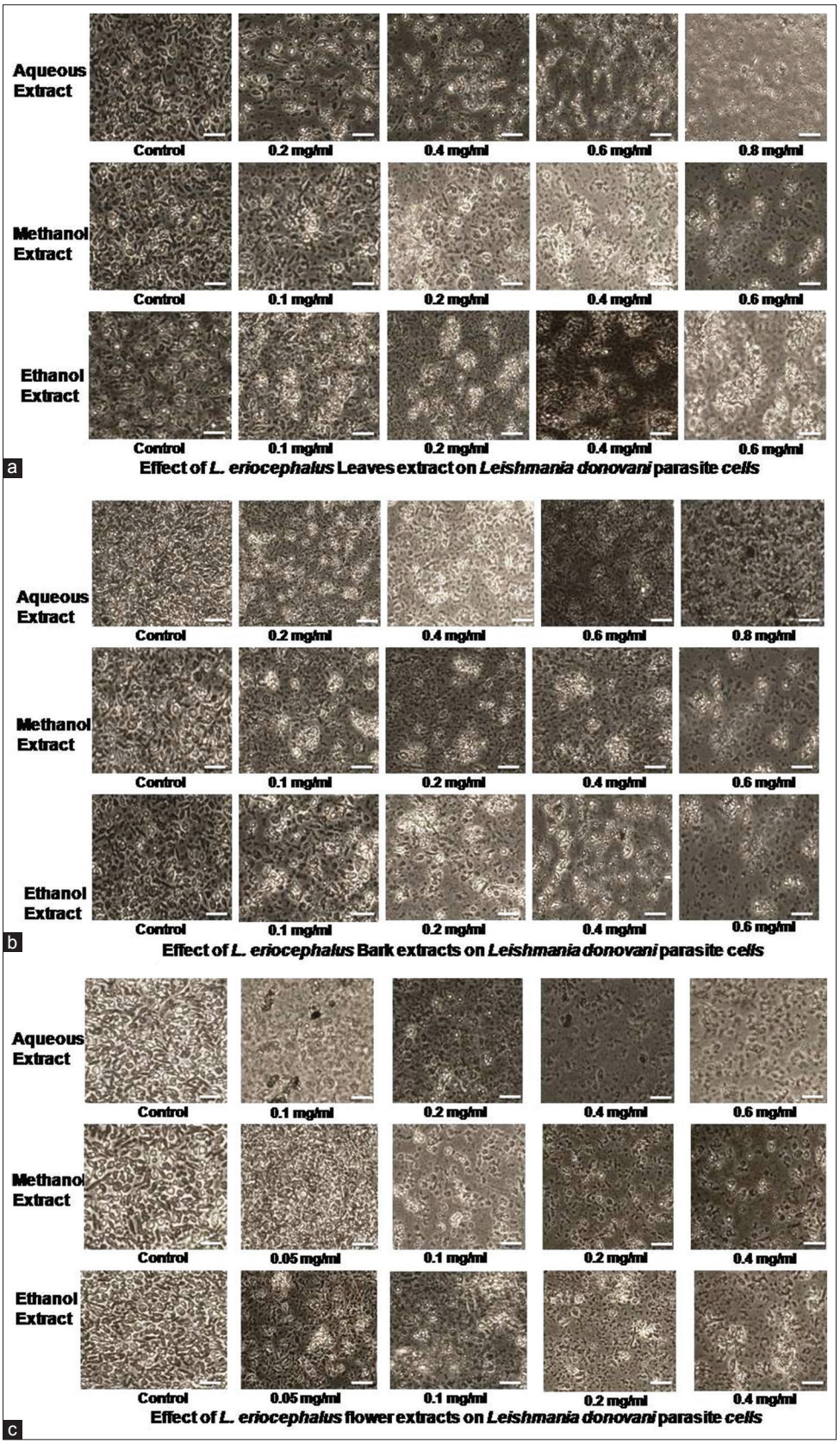

Figure 4: Cell morphology of *Leishmania donovani* cells after 24 h treatment of aqueous, methanol and ethanol extracts of (a) leaves, (b) stem bark, and (c) flowers of *Lasiosiphon eriocephalus* at different concentrations. All images are taken at ×20 magnification with Carl Zeiss phase contrast microscope. Scale bars, 100 μm

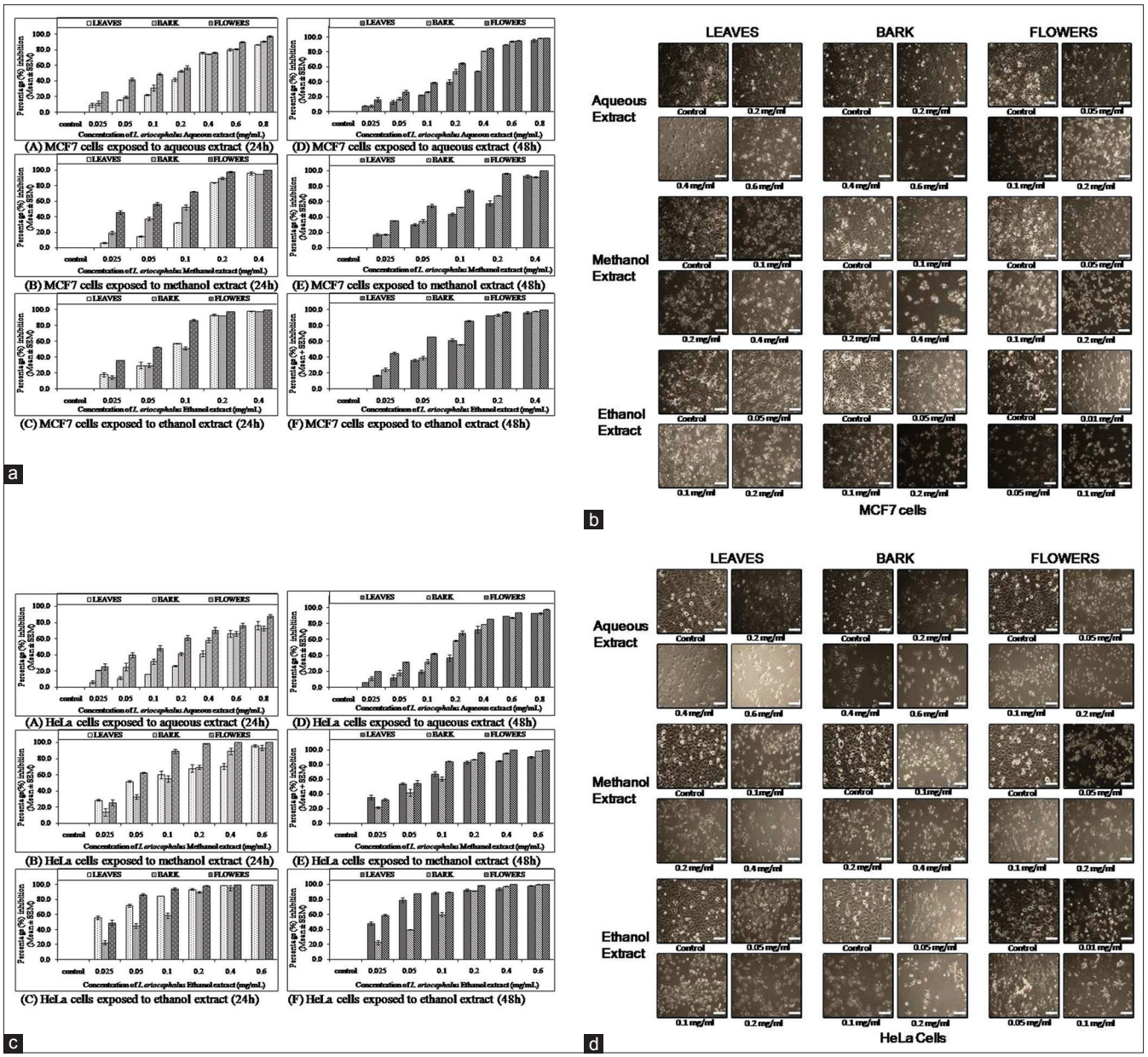

Figure 5: (a) Representative histogram showing *in vitro* dose-dependent cytotoxicity of (A) aqueous extract of leaves, bark, and flowers on MCF-7 cells exposed for 24 h, (B) MCF-7 cells exposed to methanol extracts of plant parts exposed for 24 h, (C) MCF-7 cells exposed to ethanol extracts for 24 h, (D) MCF-7 cells exposed to aqueous extracts of plant parts exposed for 48 h, (E) MCF-7 cells exposed to methanol extracts of plant parts exposed for 48 h (F) MCF-7 cells exposed to ethanol extracts of plant parts exposed for 48 h. The results represent the means of three independent experiments, and error bars represent the standard error of the mean. (b) Cell morphology of MCF-7 cells after 48 h treatment of aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers of *Lasiosiphon eriocephalus* at different concentrations. All images are taken at ×20 magnification with Carl Zeiss phase contrast microscope. Scale bars, 100 μm. (c) Representative histogram showing *in vitro* dose dependent cytotoxicity of (A) aqueous extract of leaves, bark and flowers on HeLa cells exposed for 24 h, (B) HeLa cells exposed to methanol extracts of plant parts exposed for 24 h, (C) HeLa cells exposed to ethanol extracts for 24 h, (D) HeLa cells exposed to aqueous extracts of plant parts exposed for 48 h, (E) HeLa cells exposed to methanol extracts of plant parts exposed for 48 h (F) HeLa cells exposed to ethanol extracts of plant parts exposed for 48 h. The results represent the means of three independent experiments, and error bars represent the standard error of the mean. (d) Cell morphology of HeLacells after 48 h treatment of aqueous, methanol, and ethanol extracts of leaves, stem bark, and flowers of *Lasiosiphon eriocephalus* at different concentrations. All images are taken at ×20 magnification with Carl Zeiss phase contrast microscope. Scale bars, 100 μm

tested isolates as compared to aqueous extracts of selected plant parts. The results obtained from this study indicated that the plant extracts showed the stronger antimicrobial activity than the commercially available antibiotics. When we compared antiparasitic activity of different parts of *L. eriocephalus*, earlier evaluations of few genera of *Thymelaeaceae* including *Gnidia krasssianus* and *G. cuneata,* showed the absence of antiparasitic activities.[25] In contrast to those findings, our results showed

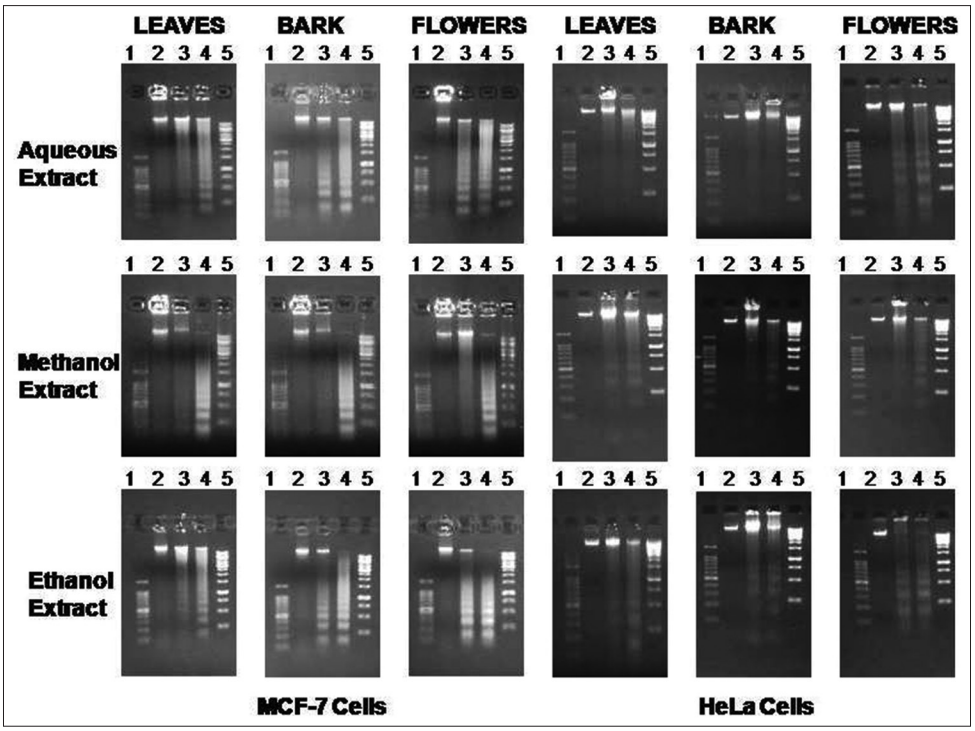

Figure 6: Representative agarose gel images showing DNA fragmentation in MCF-7 cells (Left panel) and HeLa cells (Right panel) treated with aqueous, methanol, and ethanol extracts of leaves, bark, and flowers of *Lasiosiphon eriocephalus* at IC_{50} and IC_{90} concentrations. In each representative gel, lane 1 is 100 bp DNA marker: Lane 2 is DNA from control cells followed by lane 3: DNA from IC_{50} concentration treated cells, lane 4: DNA from IC_{90} treated cells and lane 5: Is 1 kilo base pair DNA marker

strong antiparasitic properties activity of different solvent extracts of *L. eriocephalus* against *L. donovani*.

In this study, cytotoxicity of the plant parts of *L. eriocephalus* leaves, stem bark, and flowers was also investigated on MCF-7 and HeLa cells. Analyses of the cytotoxicity of the ethanol extract of flowers and leaves demonstrated considerable activity as exhibited by an IC_{50} value of 0.033 mg/mL and 0.061 mg/mL, followed by the activity of ethanol extract of stem bark with IC_{50} 0.084 mg/mL. The methanol extract of flowers showed strong cytotoxic activity with IC_{50} 0.05 mg/mL whereas the cytotoxic effects of methanol extracts of leaves and stem bark showed comparatively lower cytotoxic effects on MCF-7 cells with IC_{50} valves of 0.150 mg/mL and 0.108 mg/mL, respectively. Comparatively, the doses of aqueous extracts of leaves, stem bark, and flowers required to kill both HeLa and MCF-7 cells are higher with IC_{50} Leaves: 0.523 mg/mL, Stem bark: 0.327 mg/mL, and Flowers: 0.128 mg/mL for HeLa cells and IC_{50} Leaves: 0.313 mg/mL, Stem bark: 0.210 mg/mL, and Flowers: 0.144 mg/mL for MCF-7 cells. The crude ethanol extract of the leaves gave the highest efficacy toward HeLa cells with a half maximal inhibitory concentration of 0.024 mg/mL, which was followed by the flowers and stem bark extracts which gave IC_{50} values of 0.034 and 0.083 mg/mL, respectively. The methanol extract of flowers and leaves were moderately cytotoxic and was able to decrease cell viability by 50% at 0.047 mg/mL and 0.066 mg/mL, respectively, whereas methanol extract of stem bark showed comparatively lower doses for cytotoxicity for HeLa cells. The results clearly indicated that both methanol, as well as ethanol extracts of leaves, stem bark, and flowers, showed significant inhibitory effects on the tested HeLa as well as MCF-7 cells at comparatively lower concentrations than the aqueous extracts. A number of studies have noted cytotoxic activities of traditional medicinal plants which had not been reported before.[33-35] In our experiments, the MCF-7 and HeLa cells when treated with both aqueous extracts, methanol, and ethanol extracts for 48 h, the results showed dose-dependent cytotoxicity of leaves, stem bark, and flower extracts against tested cells. We further analyzed the DNA fragmentation, considered as one of the hallmarks of cell apoptosis was confirmed resolving DNA extracted from MCF-7 and HeLa cells exposed to IC_{50} and IC_{90} concentrations of aqueous, methanol as well as ethanol extracts of leaves, stem bark, and flowers on agarose gels. Extensive DNA double-strand breaks appeared from MCF-7 and HeLa cells exposed to plant extracts which serve as the powerful genotoxic potential of *L. eriocephalus*. Thus, our study provides plausible evidence supporting *L. eriocephalus* plant extract exhibited strong cytotoxic and apoptotic potential against selected cancer cells. Further detailed studies are necessary to enhance the molecular mechanism responsible for cytotoxicity, antiproliferative, and apoptotic effects of genotoxic compounds in this plant.

Conclusion

In the present study, ethanol extract of flowers and leaves of *L. eriocephalus* showed maximum antioxidant, antibacterial,

antiparasitic, and cytotoxic activity. The phytochemical analysis shows the presence of flavonoids, tannins, and phenols with high antioxidant activity. Antibacterial activity of the flowers, as well as leaves and stem bark, shows the ability to inhibit the growth of Gram-positive as well as Gram-negative bacteria. The cytotoxicity of different solvent extracts on cancer cell lines showed strong cytotoxic and genotoxic effects. The obtained results could form a good basis for further investigation in the potential discovery of new natural bioactive compounds and molecular mechanism involved in cytotoxicity and genotoxicity of those bioactive compounds from this traditional plant with medicinal value.

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Conflicts of interest

There are no conflicts of interest.

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