RESEARCH ARTICLE

A Comparative Screening of Medicinal Plant Extracts for Potential Antibacterial and Anticancer Properties

Thara N.K[1,2] and Raghavendra M.P[1*]

DOI: 10.18811/ijpen.v11i01.18

Abstract

The primary aim of the current study was to screen *Cuscuta chinensis* Lam., *Dendrophthoe falcata* (L.f.) Ettingsh, *Clitoria ternatea* L., *Acacia nilotica* L., and *Aristolochia littoralis* Parodi plants on a comparison basis for their antibacterial and anticancer activities. To achieve this, aqueous, chloroform, and ethanol extracts of leaves and seeds were prepared and subjected to antibacterial activity against multidrug-resistant clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. It was observed that aqueous and ethanol extracts of *A. littoralis* Parodi seeds were found to possess significant antibacterial activity against test bacteria compared to extracts obtained from leaves. Further, phytochemical analysis was performed for the extracts of each plant to determine the phytoconstituents present. The phytochemical analysis of different extracts from the seeds of all five plants revealed the presence of metabolites such as alkaloids, steroids, carbohydrates, terpenoids, tannins, and resins. Consequently, the seed extracts were investigated for anticancer activity against human skin carcinoma A431 cell lines by MTT assay. Here too, *A. littoralis* extracts showed significant anticancer activity and the other four plants had negligible or no activity. In conclusion, in the current study, it was determined that extracts of *A. littoralis* seeds, among the five medicinal plants screened, possessed both antibacterial and anticancer activity. Based on our previous study, the anticancer activity of *A. littoralis* seed extract recorded in the current study is attributed to a bioactive principle termed naneoicglycolate.

Highlights

- Five traditional medicinal plants were screened for their antibacterial and anticancer activities.
- Phytochemical analysis was performed for the extracts of the plants to determine their phytoconstituents.
- Antibacterial activity was subjected against multidrug-resistant clinical isolates of *Escherichia coli* and *Staphylococcus aureus*.
- Seed extracts of potentially screened plants were investigated for anticancer activity against human skin carcinoma A431 cell lines by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

Keywords: Phytochemicals, Medicinal plants, Extracts, Cytotoxicity, Antibacterial , Anticancer

International Journal of Plant and Environment (2025); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Plants with medicinal properties have been used as a source of medicine in many parts of the world including India such as Africa, Australia, Brazil, and China. Herbal medicines are carefully evaluated based on their safety, efficacy, and efficiency Khalid *et al*., (2022). The use of traditional plants as medicine is an age-old skill and knowledge that is practiced based on former theories and experiments conducted for maintaining health and determining the ways and means of treatment for various physical and mental illnesses using those medicinal herbs Benzie *et al*., (2011). Research suggests that xenobiotic drugs cause adverse and toxic side effects. Long-term usage of such drugs causes serious side effects leading to health complications. The development of resistance against synthetic drugs like antibiotics among bacteria is also a challenge. Hence, there is an urge for the discovery of new therapeutics from safe and efficient sources. Besides, an increasing recent preference among consumers for herbal products against synthetic medicines suggests the need for investigation of natural and traditional plants for potential therapeutics.

Medicinal plants contain numerous phytochemicals that can potentially be used for therapeutic purposes or to discover useful drugs as an alternative to their synthetic counterparts Sofowora *et al*., (2013). Researchers are interested in drug resistance as a major challenge and thus putting unprecedented efforts over the past decades to address it. Vast research is going on in search of the best alternate substances from these plants to overcome health hazards (Mahomoodally 2013). Reports suggest that more than 80% of the population in developing countries depend on wild medicinal plants to treat gut issues, infections, reproductive problems, respiratory diseases, wound healing, and pain management with their antimalarial, antibacterial, antifungal, and antiviral activities (Mahomoodally 2013). Yet, numerous studies are required to explore and apply these indigenous plants and their derivatives for lethal diseases like infectious diseases and cancers (Mahomoodally 2013).

Incidence of cancer has become the primary cause of death in both developed and developing countries leading to incessant

[1*]Department of Microbiology, Government Science College (Autonomous), Hassan, Karnataka, India.

[2]Research and Development Centre, Bharathiyar University, Coimbatore 641046, Tamil Nadu, India.

***Corresponding author:** Raghavendra M.P, Department of Microbiology, Government Science College (Autonomous), Hassan, Karnataka, India., Email: mpraghavendra@gmail.com

How to cite this article: Thara, N.K., Raghavendra, M.P. (2025). A Comparative Screening of Medicinal Plant Extracts for Potential Antibacterial and Anticancer Properties. International Journal of Plant and Environment. 11(1), 164-174.

Submitted: 08/10/2024 **Accepted:** 24/01/2025 **Published:** 28/03/2025

economic loss throughout the world due to its cumulative frequency results. The adverse effects caused to the human body by treating cancer with chemotherapy, radiotherapy, and surgery include alopecia, nausea, and vomiting. Hence, there is a great demand and urge for the discovery of novel medicinal plant species with less or no toxic effects on healthy host cells but should be more effective against cancerous cells Abdelhamed *et al*., (2013). Several studies have documented that the use of certain plant bioactive principles is not only helpful in treating cancer but is also effective in preventing various cancers Khanna (2008); Abu-Dahab *et al*., (2012). The application of these molecules is not only cheaper and safer but also has various beneficial effects due to its numerous phytochemicals Alves-silva *et al*., (2017); Gezici *et al*., (2019).

With this background, in the present study, we have screened five plants namely *Cuscuta chinensis* Lam., *Dendrophthoe falcata* (L.f.) *Ettingsh, Clitoria ternatea* L., *Acacia nilotica* L., and *Aristolochia littoralis* P. to decipher one such potential plant with both antibacterial and anticancer properties. To achieve this, aqueous, chloroform, and ethanol extract from their leaves and seeds were screened. Antibacterial activity was screened against antibiotic-resistant isolates of *Escherichia coli,* and *Staphylococcus aureus,* available in our repository. Anticancer activity was tested against human skin carcinoma A431 cell lines by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Upon comparison, *A. littorais* parodi seed extract was found to be more effective for anticancer activity and antibacterial activity than its leaf extract. In our previous study, the presence of an active molecule termed naneoicglycolate in the seed extract was reported and it was attributed to the observed antibacterial and anticancer properties (Thara and Raghavendra, 2022).

Materials and Methods

Collection and authentication of plant material

The five plants namely *C. chinensis* Lam., *D. falcata* (L.f.) Ettingsh, *C. ternatea* L., *A. nilotica* and *A. littoralis* (Table 1) which are generally considered as traditional plants were randomly collected from the Western Ghats regions of Karnataka, India. Upon taxonomical authentication for their unambiguous identity, the plants were submitted to Government Science College, Hassan, Karnataka, India. Each plant was analyzed for its phytochemical constituents. Further, all plant extracts were tested for antibacterial and anticancer activity to select potential plants with effective activity.

Extraction Process

Extraction from leaves

Fresh leaves were collected from all the 5 plants. The leaves were cleaned thoroughly with sterilized water. About 250 g of each leaf sample was weighed and ground using a pestle and mortar to get a fine paste. This paste extraction was done with three different solvents measuring 200 mL such as distilled water, 70% ethanol and chloroform, mixed thoroughly for 15 minutes at room temperature and filtered using muslin cloth (Azmir *et al*., 2013). This preparation was kept in a water bath to get a fine powder which was stored at -20°C for future use. Whenever required, the powder was dissolved in dimethyl sulfoxide (DMSO) with a final concentration of 1-mg/mL.

Extraction from seeds

Dried seeds were powdered to a coarse consistency. About 250 g of dried seeds were separated with three different solvents such as distilled water, 70% ethanol and chloroform. The seed sample was divided into three parts and treated with 150 mL of the above-mentioned solvents in separate cork bottles and kept in the shaker for 3 to 4 days at room temperature. After three days, the samples were filtered using Whatman filter paper. The extracts were kept in a water bath to get a fine powder which was stored at -20°C for future use. Whenever required, the powder was dissolved in DMSO with a final concentration of 1-mg/mL.

Soxhlet extraction

Soxhlet extraction was performed using distilled water, 70% ethanol and chloroform in classical Soxhlet apparatus. Approximately, 100 mg of seed powder of each plant was placed inside a thimble in a filter paper which was loaded into the main chamber of soxhlet extractor. The soxhlet extractor was placed into a flask containing 100 mL of each solvent separately. This cycle is repeated for 2 days. The collected mixture was placed in a water bath to remove the solvent. After the extraction process, the suspension was filtered using Whatman no. 1 filter paper and was concentrated by a rotary vacuum evaporator. The vacuum-dried extracts were preserved in the dark until further use Zhang *et al*., (2018).

Antibacterial Activity of Plant Extracts using the cup diffusion method

Bacterial isolates *E. coli* U15 and *S. aureus* P37, isolated from urine and pus samples, respectively, were selected from the repository of Government Science College, Hassan.

Antibiogram Study

The antibiogram study was performed to determine the antibiotic susceptibility profile for the clinical isolates of *E. coli* and *S. aureus*. The antibiotics used in this study were azithromycin (AZM - 15 µg), cephalothin (CEP – 30 µg), ciprofloxacin (CIP - 5 µg), cefuroxime (CXM – 30 µg), cefoxitin (CX - 30 µg), cefazolin (CZ - 30 µg), lomefloxacin (LOM – 10 µg), methicillin (MET – 5 µg), Ofloxacin (OF - 5 µg), polymyxin-B (PB – 30 µg), pefloxacin (PF – 5 µg), (Piperacillin PI - 100 µg) and tobramycin (TOB - 10 µg). All the antibiotics were used in the format of discs (HiMedia, India). Briefly, each bacterial strain was spread on the Muller Hinton agar (MHA) (HiMedia, India) plate for inoculation and the antibiotic discs were aseptically placed on the plate. Further, these culture plates were incubated at 37°C for 16 to 18 hours. After incubation, the diameter of the ZOI around the disc was measured and the bacteria showing resistance to differernt antibiotics were taken for the study. Accordingly, multidrug-resistant strains such as *E. coli* and *S. aureus* were selected in the study for further experiments.

Cup diffusion method

The method was used to screen the antibacterial activity of each plant extract using MHA plates. About 0.5 mg of water, ethanol

Table 1: Description of plants used in the study

S. No.	Plant	Common name	Family	Part used	Applications
1.	*Cuscuta chinensis*	Chinese Dodder, Akasha balli in Kannada	*Cuscutaceae*	leaves, seeds, barks, roots and stem	Antiproliferative activity, antioxidant activity, anti-inflammatory, hepatoprotective, antimicrobial, anti-osteoporotic, anxiolytic activity, anti-aging, antiviral, antibacterial, antiulcer, especially in ovarian and breast cancer, pain reliever, tumors, scabies, eczema, jaundice, fever, chest pain, itching, diarrhea, lactation, edema, stomach ache, measles, sprain, alleviation of high blood pressure, leucorrhoea, obesity, migraine, amnesia, epilepsy, constipation, sexual impotence, vision weakness, kidney and liver deficiency (Ahmad *et al*., 2017; Deepakkumar *et al*., 2017; Wang *et al*., 2021)
2.	*Dendrophthoe falcata*	Honey Suckle Mistletoein English and Banda in Hindi, Maduk badanike in Kannada	*Loranthaceae*	leaves, seeds, barks, roots, stem and flowers	Curing wounds, ulcers, pulmonary tuberculosis, renal and vesical calculi, asthma, swelling, menstrual disorders, kapha, pitta. Cooling, bitter, astringent, aphrodisiac, antipyretic, antiallergic, narcotic, acts as anti-microbial, anti-oxidant, antiseptic, antibiofilm, anxiolytic, cytotoxic, anti-tumor, antidiabetic, hepatoprotective, antifungal, thrombolytic, immunostimulatory, antihyperlipidemic, anti-fertility, anti-cancer, antibacterial (Kumar *et al*., 2023; Rafe *et al*., 2015; Endharti *et al*., 2016)
3.	*Clitoria ternatea*	bluebell vine, blue pea, butterfly, pea, cordofan pea or Darwin pea, Sattaga in Kannada	*Fabaceae*	leaves, stem and flowers	Memory enhancing, antistress, nootropic, antidepressant, anxiolytic, anticonvulsant, antibiofilm, antioxidant, sedative and tranquilizing properties, antimicrobial, anti-inflammatory, antipyretic, analgesic, anesthetic, diuretic, anticancer, insecticidal, antidiabetic (Jacob *et al*., 2013; Jeyaraj *et al*., 2022; Alshamrani *et al*., 2022)
4.	*Acacia nilotica*	*Vacellia nilotica*, gum Arabic tree, thorn mimosa, thorny acacia or babul, Naachike mullu in Kannada	*Fabaceae*	Leaves, stems, flowers, fruits and twigs	Food, fodder, lumber, medicines for dysentery, piles, stomach ulcers, tuberculosis, smallpox, leprosy, cough, ophthalmia, skin cancer, toothache, antimicrobial, antioxidant, anticancer, antileishmanial, antiamastigote, antifungal, antiviral and immunomodulatory (Revathi *et al*., 2017; Abdalla *et al*., 2020; Ahamed *et al*., 2023; Diab *et al*., 2022)
5.	*Aristolochia littoralis*	Dutchman's pipe, Eshwariballi in Kannada	*Aristolochiaceae*	Seed, root, leaves, stem, flower and twigs	Treat rheumatism, arthritis, myocardial infarction, urinary tract cancer, renal interstitial fibrosis, muscle relaxants, cholera, abdominal pain, skin problems, stings, bites of insects and animals, heart and liver protector, bronchitis, constipation, bladder diseases, healing of wounds, pain relievers and angina, antimicrobial, anticancer, analgesics, anti-inflammatory, antioxidant, antimalarial, antidotes for snake bites, antihistamines, antiparasitics, angiogenic, antinociceptive, antifibrosis, nephroprotection, neuroprotective, antiulcer, antiallergic, antidiabetic, antihemorrhagic, antispasmodic, antitoxin and antiplatelet (Lerma-Herrera *et al*., 2022; Salome *et al*., 2020; Montiel-Ruiz *et al*., 2020)

and chloroform extract of each plant was dissolved in 10 mL of distilled water to make 50 µg/mL of stock concentration. From this stock, further 40, 30, 20, 10 and 5 µg/mL concentrations were prepared using distilled water. Using a sterile cork borer, six equidistant wells were made on the MHA plates. A lawn of each test organism was plated on the agar using a sterile swab as mentioned earlier. All the wells were labeled and wells were loaded with 100 µL of different concentrations of the extracts along with 100 µL azithromycin (50 µg/mL) as standard. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition (ZOI) around each well was measured in millimeters (mm) (Sastry and Bhat 2018).

Phytochemical Analysis

Preliminary phytochemical analysis was carried out for each plant extract to identify different secondary metabolites present and to assess the presence or absence of different phytochemicals. The presence of alkaloids was examined using Dragendroff's test, Wagners's test, Mayer's test and Hager's test for the aqueous, ethanol and chloroform extracts prepared from

all 5 plants. The presence of carbohydrates in the extracted aqueous, ethanol and chloroform extracts, prepared from the five selected plants, was tested by Molisch's test, Fehling's test and Benedict's test. Steroid activity was examined by the Libermann-Burchard and Salkowski test for all three extracts. Similarly, all the extracts were tested for saponins, tannins by ferric chloride test, flavonoids by Shinoda's test, phenols, coumarins, triterpenoids, carboxylic acid, resin and quinine Harborne (1984), Harborne (1998); Rufai *et al*., (2016), Kancherla *et al*., (2019).

Anticancer Activity

Based on the phytochemical constituents and antibacterial activity seed extracts from all five plants were selected for evaluation of anticancer activity using MTT assay Mosmann (1983); Cole (1986); Van Meerloo *et al*., 2011. Briefly, the A431 skin cancer cell line was procured from ATCC and cultured in DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). Cell viability was determined and the cells were counted. Further, 50,000 cells/well were seeded in a 96-well plate and incubated for 24 hours at 37°C, in a 5% CO_2 incubator. For *in vitro* cytotoxicity, the monolayer cell culture was trypsinized and the cell count was adjusted to 1 x 10^5 cells/mL using respective media containing 10% FBS. To each well, 100 µL of the diluted cell suspension (50,000 cells/well) was added and incubated to form a monolayer of cells. After incubation, the supernatant was removed and the cell monolayer was washed with the growth medium. Further, the seed extracts of all five plants (distilled water, chloroform, and ethanol method) were used as test samples and a well with only growth medium without extract was used as control. About 100 µL of different concentrations of the test sample ranging from 10 to 320 µg/mL was added to the partial monolayer and the plates were incubated at 37°C for 24 hours in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µL of MTT (5 mg/10 mL of MTT in PBS) was added to each well followed by incubation for 4 hours at 37°C in a 5% CO_2 atmosphere. Following incubation, the supernatant was removed 100 µL of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and the concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values was generated from the dose-response curves for each cell line. The IC_{50} values were derived from a nonlinear regression analysis (curve fit) based on a sigmoid dose-response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, SanDiego, CA, USA).

$$\%\ Inhibition = 100 - \frac{[OD\ of\ sample]}{[OD\ of control]}\ x\ 100$$

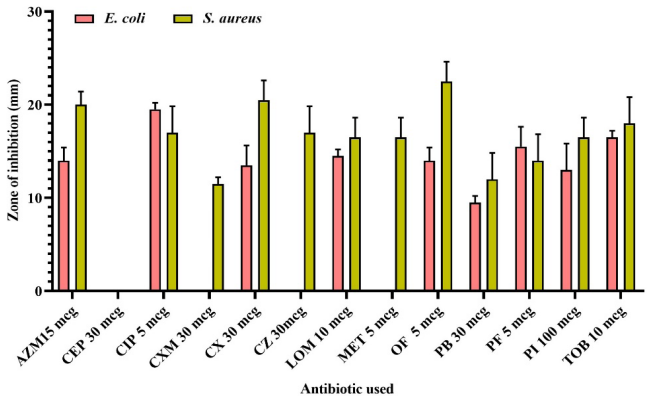

Figure 1: Antibiogram study for the different antibiotics against *E. coli* and *S. aureus*

Results

Antibacterial Activity of Plant Extracts using the Cup Diffusion Method

Based on the antibiogram study the clinical isolates of *E. coli* and *S. aureus* were found to be multidrug-resistant strains (Fig. 1). Further, both leaves and seed extracts of five plants were subjected to antimicrobial activity against those clinical isolates of *E. coli,* and *S. aureus*. Irrespective of the plant and extraction method, the extracts from leaves had no or negligible inhibitory activity against the clinical isolates tested. On the other hand, seed extracts demonstrated readable inhibitory activity against both *E. coli* and *S. aureus* (Table 2). Upon thorough comparative analysis of all, only the seed extracts (ethanol and aqueous) from *A. littoralis* showed significant antibacterial activity.

Phytochemical Analysis

Phytochemical analysis was performed for the extracts obtained from all five plants to know the different chemical constituents present in these plants (Table 3). The result showed the presence of assorted phytochemicals in these plants. Alkaloids, steroids, carbohydrates, tannins, terpenoids, phenols, and resins were present in all five plants. Amino acids, flavonoids, and coumarins were present in all four plants except *A. littoralis*. Carboxylic acid was present only in *C. chinensis, C. ternatea* and *A. nilotica* whereas, quinones were present only in *D. falcata* and *C. ternatea*

Anticancer Activity of *A. littoralis* Parodi Seed Extracts

To assess the cytotoxic activity of the seed extracts from five plants, an MTT assay was performed with increasing concentrations of different solvent extracts. The obtained results indicated that only *A. littoralis* demonstrated concentration-dependent anticancer activity irrespective of the method of extraction. With increasing concentration of the extract, anticancer activity was also found to be incremental, the highest being at 320 µg/mL. Among the different extracts tested, chloroform extract recorded a highly significant cytotoxic activity compared to control along with aqueous extract followed by ethanol extract (Fig. 2). The microscopic observation also revealed the high anticancer activity at higher concentrations of extracts compared to lower as well as control (Fig. 3). Further, IC_{50} values were obtained to be 238, 239.4, and 118.9 µg/mL for distilled water, chloroform, and ethanol extracts, respectively (Table 4).

Table 2: Antibacterial activity of seed extracts from all five plants against multidrug-resistant clinical isolates of *E. coli* and *S. aureus*

Plant	*Type of extract*	*Organism*	*The zone of inhibition measured in mm concentration in µg/mL*					
			50	*40*	*30*	*20*	*10*	*5*
C. chinensis	Chloroform	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Ethanol	E. coli	R	R	R	R	R	R
		S. aureus	9 ± 0.10	R	R	R	R	R
	Distilled water	E. coli	R	R	R	R	R	R
		S. aureus	13 ± 0.15	R	R	R	R	R
D. falcata	Chloroform	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Ethanol	E. coli	R	R	R	R	R	R
		S. aureus	9 ± 0.21	R	R	R	R	R
	Distilled water	E. coli	R	R	R	R	R	R
		S. aureus	9 ± 0.11	9 ± 0.10	R	R	R	R
C. ternatea	Chloroform	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Ethanol	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Distilled water	E. coli	R	R	R	R	R	R
		S. aureus	14 ± 0.10	7 ± 0.11	9 ± 0.21	5 ± 0.12	6 ± 0.23	R
A. nilotica	Chloroform	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Ethanol	E. coli	R	R	R	R	R	R
		S. aureus	7 ± 0.11	R	R	R	R	R
	Distilled water	E. coli	R	R	R	R	R	R
		S. aureus	9 ± 0.15	18 ± 0.11	16 ± 0.23	R	R	R
A. littoralis	Chloroform	E. coli	R	R	R	R	R	R
		S. aureus	R	R	R	R	R	R
	Ethanol	E. coli	R	R	8 ± 0.12	R	R	R
		S. aureus	20 ± 0.21	10 ± 0.33	10 ± 0.11	10 ± 0.15	16 ± 0.15	R
	Distilled water	E. coli	R	R	R	R	R	R
		S. aureus	8 ± 0.10	8 ± 0.15	10 ± 0.21	8 ± 0.11	8 ± 0.11	8 ± 0.11

Note: R: Resistant, values are mean of triplicates ± SE

Discussion

With the increasing awareness about the toxic effects associated with chemical and synthetic additives ranging from antioxidants, flavor agents, antimicrobials, anticancer drugs, and such among the civic population, the need for natural alternative products is increasing. To address this pressing need, researchers are extensively exploring natural plant extracts for promising active molecules that can replace their synthetic counterparts by demonstrating an extensive range of properties including antimicrobial, anticancer, antioxidant, and flavor enhancement Ashraf (2020); Nejhad *et al*., (2023).

One such immediate look is for natural antimicrobials and anticancer drugs from plant sources. This is because of the rise in the incidence of infectious diseases worldwide. Additionally, bacterial infections have become a significant health problem due to the emergence of multidrug-resistant bacterial species Tarfaoui *et al*., (2022). As per the US Centre for Disease Control and Prevention (CDC), nearly 2 million Americans have contracted antibiotic illnesses, and around 40,000 fatalities and illnesses

Table 3: Phytochemical analysis of different extracts of all five plants

Phytochemical constituents	*C. chinensis*						*D. falcata*						*C. ternatea*						*A. nilotica*						*A. littoralis*					
	Leaf			*Seed*			*Leaf*			*Seed*			*Leaf*			*Seed*			*Leaf*			*Seed*			*Leaf*			*Seed*		
	E	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*	*E*	*C*	*AE*
Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+
Steroid	+	-	-	+	-	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Terpenoid	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	+
Coumarins	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-	+	-	-	+	-	-	+
Carboxylic acid	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
Amino acids	+	-	+	+	-	+	+	+	-	+	+	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-
Resin	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Quinone	-	-	-	-	-	-	+	-	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

+ Present; - absent; E- Ethanol; AE- Aqueous extract; C- Chloroform extract

Table 4: Cytotoxic activity of *A. littoralis* seed extracts against A431 cell lines

Extracts	*Concentration (µg/mL)*	*OD at 590 nm*	*%Inhibition*	*IC_{50} µg/mL*
Control	0	0.6159	0.00	-
Distilled water	10	0.599	2.81	238
	20	0.569	7.66	
	40	0.521	15.46	
	80	0.436	29.26	
	160	0.396	35.66	
	320	0.250	59.34	
Chloroform	10	0.596	3.31	239.4
	20	0.574	6.82	
	40	0.541	12.16	
	80	0.457	25.78	
	160	0.328	46.76	
	320	0.225	63.47	
Ethanol	10	0.602	2.21	118.9
	20	0.5741	6.79	
	40	0.527	14.40	
	80	0.431	29.96	
	160	0.329	46.60	
	320	0.263	57.35	

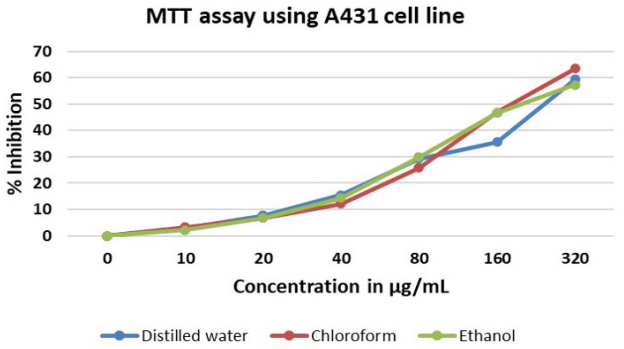

Figure 2: Cytotoxicity activity of *A. littoralis* extracts on A431 cell lines at different concentrations

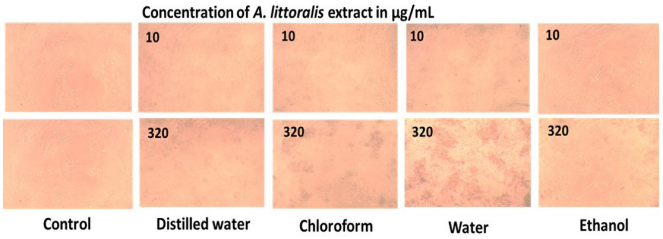

Figure 3: Microscopic observation of A431 cells treated with different concentrations of *A. littoralis* extracts. Cytotoxic effect/cell death/apoptosis was in an increasing trend with the increase in the concentration of the extract

in Europe were due to multidrug-resistant bacteria Altun *et al*., (2018); CDC 2022). The exploration of traditional plants for their antimicrobial and anticancer properties has garnered significant scientific interest, as these botanicals are rich sources of bioactive compounds (Aarestrup (2005); Rai and Lalramnghinglova (2010); Wang *et al*., (2012); Teoh (2016). Numerous studies have documented the efficacy of various traditional plants, revealing that phytochemicals such as alkaloids, flavonoids, and terpenes possess notable antimicrobial and anticancer activities Kennedy *et al*., (2011); Khan *et al*., (2019); Ashraf *et al*., (2022). By leveraging the wisdom embedded in traditional medicine, researchers aim to isolate and characterize these active constituents, potentially leading to the development of new therapeutic agents that address pressing health challenges such as antibiotic resistance and cancer (Wang and Weller (2006); Verma *et al*., (2018). This integrative approach not only highlights the importance of biodiversity in drug discovery but also underscores the potential for sustainable, plant-based solutions in modern medicine. In the present study, we examined five plants namely *C. chinensis* Lam., *D. falcata* (L.f.) Ettingsh, *C. ternatea* L., *A. nilotica* L and *A. littoralis* P. to decipher one such potential plant with both antibacterial and anticancer properties. The study was designed as a comparative analysis of the five plants to determine the most promising one to demonstrate both antibacterial and anticancer activity against multidrug-resistant bacterial strains and skin cancer cell lines, respectively.

C. chinensis is a parasitic plant with significant medicinal importance in Chinese traditional medicine. It is renowned for its potential therapeutic properties, particularly improving liver and kidney function, supporting reproductive health, and enhancing vitality. Traditionally, the herb *C. chinensis* in the form of decoction or extract was consumed by people, whereas its active constituents are being used in modern medicine (Ahmad 2017). For instance, an herbal mixture derived from *Cuscuta chinensis* exhibited therapeutic effects for postmenopausal osteoporosis and ovarian cancer (Abdelhamed 2013). The crude ethanol extract of *C. Chinese* (TSZ) is known to contain osteogenic compounds and is used for the treatment of osteoporosis Yang *et al*., (2011). Overall, the medicinal significance of *Cuscuta chinensis* lies in its rich chemical composition and its longstanding role in traditional herbal practices.

D. falcata is a hemiparasitic plant with notable medicinal properties, particularly in traditional medicine across various cultures. It has been used to treat a range of ailments, including respiratory disorders, hypertension, and digestive issues. Research has highlighted the potential of *D. falcata* anti-inflammatory, antioxidant, and anticancer activities. For instance, various extracts of *D. falcata* stems are reported to possess the potential inhibitory effect on carcinoma cells (Dashora *et al*., (2011a); Dashora *et al*., (2011b); Pattanayak *et al*., (2011); Rafe *et al*., (2015); Bhagat and Kondawara (2021); Alahmdi *et al*., (2022). Some studies suggest that extracts from this plant may help boost the immune system and possess antimicrobial properties Bhagat *et al*., (2022).

C. ternatea is a plant renowned for its medicinal properties across Southeast Asia. The flowers, leaves, and roots of *C.*

ternatea are utilized for their diverse therapeutic benefits. It has been used for intellect-promoting activities Mukherjee *et al*., (2008). In a recent study, methanol extract from *C. ternatea* leaves was found to demonstrate both antibacterial against *Salmonella typhi* and anticancer activity against human promyelocytic leukemia cells indicating the possible therapeutic application of its bioactive compounds Das *et al*., (2020). Leaf and flower extracts of the *C. ternatea* plant have shown antibacterial against infectious gram-negative and gram-positive bacteria proving the plant to possess antibiofilm, antioxidant, antibacterial, and cytotoxic properties Ananda *et al*., (2011); Dhanasekaran *et al*., (2019); Islam *et al*., (2023).

A. nilotica holds significant medicinal importance in traditional and contemporary medicine. Various parts of *Acacia nilotica*, including the bark, leaves, and gum, are utilized for treating ailments such as diarrhea, dysentery, and respiratory issues. Rich in bioactive compounds, *A. nilotica* exhibits notable antimicrobial, anti-inflammatory, antidiabetic, and antioxidant properties (Sadiq *et al*., (2017); Rauf *et al*., (2024). Fruit extract of *A. nilotica* produced nanocomposites ZrO_2/RGO, flavonoids, and polyphenolics served as capping agents to maximize anticancer potential reducing its toxicity to normal cells (Al-Rajhi *et al*., (2023); Ahamed *et al*., (2023).

A. littoralis is a plant with a history of traditional medicinal use. While specific research on this species may be limited, members of the *Aristolochia* genus have been studied for their potential therapeutic properties. Dichloromethane (DCME) extract from the leaves and stem of *A. foetida* caused late apoptotic cell death in cancer cell line MCF-7 cells Lerma-Herrera *et al*., (2021). *A. ringens* ethanolic and methanolic root extracts possess significant anticancer activity for sarcoma-180, solid tumors, and lymphoid leukemia Akindele *et al*., (2014). Chloroform extract of the roots and leaves of *A. baetica* and *A. indica* demonstrated effective cytotoxic activity against MCF-7 breast cancer cells and MCF-7 cells Chaouki *et al*., (2010); Subramaniyan *et al*., (2015). The literature survey provided information regarding the antibacterial activity of plant extracts from *Aristolochia* genus against *B. subtilis, Vibrio cholerae, Klebsiella pneumoniae, Salmonella typhi* and *S. paratyphi* A, *S. flexneri Pseudomonas fluorescens, E. coli, Listeria monocytogenes* and antifungal activity against *Saccharomyces cerevisiae, Aspergillus terreus, Pennicillium notatum,* and *Rhizopus stolonifer* (Kavitha and Nirmaladevi (2009); Benarba and Meddah (2014) as well as antiviral activity against HIV (Navarro-Garcia *et al*., 2011). However, its activity against drug-resistant bacteria has not been tested. Therefore, we aimed to test its antibacterial activity against multidrug-resistant bacterial isolates such as *E. coli* and *S. aureus*.

In the present study, ethanol, chloroform, and aqueous extracts of all five selected plants were prepared to extract all important bioactive compounds in one or more extracts. With this approach, important bioactive compounds present in the plants shall be extracted and examined for their therapeutic activity. Initially, the extracts were evaluated for antibacterial activity against clinical isolates of *E. coli* and *S. aureus*, which, upon examination, were confirmed as multidrug-resistant strains. For unknown reasons, no inhibitory activity of the leaf extracts was seen, irrespective of the plant and extraction method, against the clinical isolates. Higher concentrations of the leaf extracts might inhibit bacterial growth, a hypothesis that can be tested. However, the inhibitory activity of seed extracts (aqueous and ethanol) was encountered at the concentrations tested. This inhibitory activity of seed extracts from all five plants indicated the presence of promising bioactive compounds in them. And, this was confirmed when the seed extracts were subjected to phytochemical analysis. The presence of assorted phytochemicals in the plant extracts accentuated the presence of bioactive compounds and the diversity in the plants selected. From a thorough comparative analysis of the antibacterial activity of all the seed extracts, *A. littoralis* was found to be superior among all five plants. Since only seed extracts showed antibacterial activity, the same was subjected to evaluate anticancer properties. To the best of our knowledge, this is the first study evaluating the anticancer activity of the seed extract; and chloroform extract of *A. littoralis* was found to possess that activity. In our previous study, the anticancer activity of naneoicglycolate, a purified bioactive compound of *A. littoralis* Parodi seed extract, on the A431 skin cancer cell line was reported (Thara and Raghavendra, 2022). This active molecule from *A. littorlais* was confirmed as naneoicglycolate using mass spectrometric (MS) analysis and its structure was elucidated using nuclear magnetic resonance (NMR). The quantitative apoptosis analysis of the molecule revealed a reduction of proliferating cells revealing its anticancer property. Based on that study, we attribute the anticancer activity of *A. littoralis* seed extract recorded in the current study to naneoicglycolate. However, the antibacterial activity could also be exhibited by the same bioactive compound or any other compound. It could be a different compound altogether because, all three forms of seed extracts possessed antibacterial activity and based on our earlier study, naneoicglycolate was encountered only chloroform extract. These hypotheses can only be confirmed upon further detailed study. It is conclusive from the current study, that *A. littoralis* possesses promising antibacterial and anticancer activity among the five plants screened. Yet, further research, emphasizing the need for careful evaluation and clinical studies, is needed to explore the potential benefits and risks associated with *A. littoralis* and establish safe and effective uses.

Conclusion

Among the five plants *C. chinensis, C. ternatea, A. nilotica .,D. falcate* and *A. littoralis* screened, *A. littoralis* proved to be the potential among the other four with its phytochemical constituents and antibacterial activity which was further selected for the anticancer activity. To the best of our knowledge, this study is the first of its kind on the *A. littoralis* Parodi seed extract for anticancer activity, wherein the chloroform extract exhibited the highest anticancer activity against A431 skin cancer cell line compared to its aqueous and ethanol extracts. With our supporting study reported earlier, the anticancer activity recorded in the present study is confirmatively attributed to naneoicglycolate of *A. littoralis* seeds, with possible antibacterial activity too.

Acknowledgment

The authors are thankful to Skanda Life Sciences, Bengaluru, for providing access to their instrumentation facility for conducting the present work.

Author Contribution

TNK conducted the experiments, manuscript preparation; MPR supervision and draft editing.

Conflict of Interest

The authors have no conflict of interest

References

Aarestrup, F.M. (2005). Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clinical Pharmacological and Toxicology. 96,* 271–281. 10.1111/j.1742-7843.2005.pto960401.x.

Abdalla, A.A., Mustafa, M.I., & Makhawi, A.M. (2020). Phytochemical screening and antimicrobial activities studies of *Acacia nilotica* fruit cover. *BioRxiv.* 10.1101/2020.02.11.943456.

Abdelhamed, S., Yokoyama, S., Hafiyani, L., Kalauni, S.K., Hayakawa, Y., Awale, S., & Saiki, I. (2013). Identification of plant extracts sensitizing breast cancer cells to TRAIL. *Oncology Reports. 29,* 1991–1998. 10.3892/or.2013.2293.

Abu-Dahab, R., Afifi, F., Kasabri, V., Majdalawi, L., & Naffa, R. (2012). Comparison of the antiproliferative activity of crude ethanol extracts of nine *Salvia* species grown in Jordan against breast cancer cell line models. *Phcogology Magazine.* 8, 319. 10.4103/0973-1296.103664

Ahamed, M., Akhtar, M.J., & Majeed Khan, M.A. (2023). ZrO_2 nanoparticles anchored on RGO sheets: Eco-friendly synthesis from *Acacia nilotica* (L.) fruit extract, characterization, and enhanced anticancer activity in different human cancer cells. *Materials Today Communications*. 36, 106756. https://doi.org/10.1016/j.mtcomm.2023.106756

Ahmad, A., Tandon, S., Dang Xuan, T., & Nooreen, Z. (2017). A review on phytoconstituents and biological activities of *Cuscuta* species. *Biomedicine and Pharmacotherapy. 92,* 772-795. 10.1016/j.biopha.2017.05.124.

Akindele, A.J., Wani, Z., Mahajan, G., Sharma, S., Aigbe, F.R., Satti, N., Adeyemi, O.O., & Mondhe, D.M. (2014). Anticancer activity of *Aristolochia ringens. Journal of Traditional and Complementary Medicine. 5,* 35-41. 10.1016/j.jtcme.2014.05.001

Alahmdi, M.I., Khasim, S., Vanaraj, S., Panneerselvam, C., Mahmoud, M.A.A., Mukhtar, S., Alsharif, M.A., Zidan, N.S., Abo-Dya, N.E., & Aldosari, O.F., (2022). Green Nanoarchitectonics of ZnO Nanoparticles from *Clitoria ternatea* Flower Extract for In Vitro Anticancer and Antibacterial Activity: Inhibits MCF-7 Cell Proliferation via Intrinsic Apoptotic Pathway. *Journal of Inorganic and Organometallic Polymers and Materials. 32,* 2146-2159. https://doi.org/10.21203/rs.3.rs-1269775/v1.

Al-Rajhi, A.M.H., Qanash, H., Bazaid, A.I.H., Binsaleh, N.K., & Abdelghany, T.M. (2023). Pharmacological evaluation of *Acacia nilotica* flower extract against *Helicobacter pylori* and human hepatocellular carcinoma in vitro and in silico. *Journal of Functional Biomaterials. 14,* 237. 10.3390/jfb14040237.

Alshamrani, S.M., Safhi F.A., Mobasher M.A., Saleem R.A., Alharthi A., Alshaya D.S., & Awad N.S. (2022). Antiproliferative effect of Clitoria ternatea ethanolic extract against colorectal, breast and medullary thyroid cancer cell lines. *Separations. 9,* 331. https://doi.org/10.3390/separations9110331.

Altun, I. & Sonkaya, A. (2018). The most common side effects experienced by patients were receiving first cycle of chemotherapy. *Iran Journal of Public Health. 47,* 1218–1219. PMC6123577; PMID30186799

Alves-Silva, J.M., Romane, A., Efferth, T., & Salgueiro, L. (2017). North African medicinal plants traditionally used in cancer therapy, *Frontiers in Pharmacology. 8,* 383. 10.3389/fphar.2017.00383

Anand, S. P., Doss, A., & Nandagopalan, V. (2011). Antibacterial studies on leaves of Clitoria ternatea A high potential medicinal plant. *International Journal of Applied Biology and Pharmaceutical technology. 2, 3*, 453–456. ISSN: 0976-4550

Ashraf, M. A., Sayed, S., Bello, M., Hussain, N., Chando, R.K., Alam, S., & Hasan, M.K. (2022). CDK4 as a phytochemical based anticancer drug target. *Informatics in Medicine Unlocked*, *28*, 100826.

Ashraf, M.A. (2020). Phytochemicals as Potential Anticancer Drugs: Time to Ponder Nature's Bounty. *Biomedical Research International.* 8602879. 10.1155/2020/8602879

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., & Omar, A.K.M., (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering, 117(4),* 426-436. 10.1016/j.jfoodeng.2013.01.014

Benarba, B., & Meddah, B. (2014). Ethnobotanical study, antifungal activity, phytochemical screening and total phenolic content of Algerian *Aristolochia longa*. *Journal of intercultural Ethnopharmacology*, *3*(4), 150.

Benzie, I.F.F.S., Wachtel-Galor., (2011). Herbal medicine: biomolecular and clinical aspects, in: Herbal Medicine: Biomolecular and Clinical Aspects, CRC Press/Taylor & Francis Copyright © 2011 by Taylor and Francis Group, LLC., Boca Raton (FL, 2011). PMID: 22593937.

Bhagat, V.C.A., & Kondawara, M.S. (2021). GC-MS analysis and *in vitro* cytotoxicity study of *Dendrophthoe falcata* and *Tridax procumbens* extracts against HaCat cells line. *International Journal of Pharmacology Science and Research*. 12, 4, 2175–2184. doi:10.13040/ijpsr.0975-8232.12(4).2175-84

Bhagat, V.C., Kondawar, M., Kardile, D.P., Awate, P.B., Tarke, S.R. & Sonawane, R.A., (2022). Evaluation of antioxidant, anticancer and antiparkinson's potential of dcme extracts of *Dendrophthoe falcate (L) and Tridax procumbens (*L) plants. *Neuro Quantology, 20*, 3015.

CDC. What Exactly Is Antibiotic Resistance? Available online: https://www.cdc.gov/drugresistance/about.html.

Chaouki, W., Leger, D.Y., Eljastimi, J., Beneytout, J.-L., & Hmamouchi, M. (2010). Antiproliferative effect of extracts from *Aristolochia baetica* and *Origanum compactum* on human breast cancer cell line MCF-7. *Pharmacology Biology. 48,* 269–274. 10.3109/13880200903096588

Cole, S.P.C., (1986). Rapid chemosensitivity testing of human lung tumor cells using the MTT assay. *Cancer Chemotherapy and Pharmacology, 17(3),* 259-263. 10.1007/BF00256695

Das, A., Priya, G.S., Soundariya, S., Deepesh, P., Edwin, A.R., Vihashinee, E., Rubiga, A., Megavarthini, S., Eswaran, R. & Bindhu, J., (2020). Antibacterial and in vitro anticancer study of methanol extracts of *Clitoria ternatea* leaves. *Journal of Natural Remedies. 12*, 96-102. https://doi.org/10.18311/jnr/2020/24381

Dashora, N., Sodde, V., Bhagat, J., S Prabhu, K., & Lobo, R. (2011a). Antitumor activity of *Dendrophthoe falcata* against *ehrlich ascites* carcinoma in Swiss albino mice. *Pharmacology Crops. 2 (1).* 1–7. doi:10.2174/2210290601102010001

Dashora, N., Sodde, V., Prabhu, K. S., & Lobo, R. (2011b). *In vitro* cytotoxic activity of *Dendrophthoe falcata* on human breast adenocarcinoma Cells-MCF-7. *International Journal of Cancer Research. 7 (1),* 47–54. doi:10.3923/ijcr.2011.47.54

Deepakkumar, R., Sabari, E., Karthick, M., & Raysad, K.S. (2017). Traditionally used ethno-medicinal plants of the Kurumba communities surrounded in Thalamalai hills, Namakkal district, Tamil Nadu. *South Indian Journal of Biological Sciences. 3*, 15-26. 0.22205/sijbs/2017/v3/i1/110354

Dhanasekaran, S., Rajesh, S., Mathimani, T., Melvin, S., Samuel, S., Shanmuganathan, R., & Brindhadevi, K. (2019). Efficacy of crude extracts of *Clitoria ternatea* for antibacterial activity against gram negative bacterium (*Proteus mirabilis*). *Biocatalysis and Agricultural Biotechnology. 21,* 10.1016/j,bcab.2019.101328.

Diab, K.A., Fahmy, M.A., Hassan, E.M., & El-Toumy, S.A. (2022). Evaluation of the cytotoxic, anticancer and genotoxic activities of *Acacia nilotica* flowers and their effects on N-methyl-N-nitrosourea induced genotoxicity in mice. *Molecular Biology Reports. 49,* 8439-8448. 10.1007/s11033-022-07662-0

Endharti, A. T., Wulandari, A., Listyana, A., Norahmawati, E., & Permana, S. (2016). *Dendrophthoe pentandra* (L.) Miq extract effectively inhibits inflammation, proliferation and induces p53 expression on colitis-associated colon cancer. *BMC Complementation and Alternate Medicine. 16,* 374–381. doi:10.1186/s12906-016-1345-0.

Gezici, S. & Sekerouglu, N.C. (2019). Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents, Anti-Cancer Agents *Medical Chemistry. 19,* 101–111. .2174/1871520619666181224121004

Harborne, A.J., (1998). Phytochemical methods a guide to modern techniques of plant analysis. *Springer Science & **B**usiness Media*. 978-0-412-57260-9

Islam, A., Mondal, S.K., Islam, S., Shorna, N.A., Biswas, S., Uddin, S., Zaman, S., & Saleh, A. (2023). Antioxidant, cytotoxicity, antimicrobial activity and In-silico analysis of the methanolic leaf and flower extracts of *Clitoria ternatea*. *Biochemistry Research International.* 10.1155/2023/8847876

Jacob, L., & Latha, M. S. (2013). Anticancer activity of *Clitoria ternatea* linn. Against dalton's lymphoma. *International Journal of Pharmacognosy and Phytochemical Research. 4*, 107–112.

Jeyaraj, E.J., Lim, Y.Y., & Choo, W.S. (2022). Antioxidant, cytotoxic, antibacterial activities of *C. ternatea* flower extracts and anthocyanin-rich fraction. *Scientific reports. 12*, 14890. doi.org/10.1038/s41598-022-19146-z

Kavitha, D., & Nirmaladevi, R. (2009). Assessment of *Aristolochia bracteolata* leaf extracts for its biotherapeutic potential. *African Journal of Biotechnology*, *8(17).*

Kennedy, D.O. & Wightman, E.L. (2011). Herbal Extracts and Phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Advance Nutrition. 2*, 32–50. 10.3945/an.110.000117

Khalid, M., Amayreh, M., Sanduka, S., Salah, Z., Al-Rimawi, F., Ghassab, M., Al-Mazaideh., Abdulkareem, A., Alanezi., Wedian, F., Alasmari, F., & Shalayel, M.H.F. (2022). Assessment of antioxidant, antimicrobial, and anticancer activities of Sisymbrium officinale plant extract. *8*, 1. https://doi.org/10.1016/j.heliyon.2022.e10477

Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S.A., Afridi, S., & Shinwari, Z.K. (2019). Anticancer Plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. *Biomolecules*. *10,* 47. 10.3390/biom10010047.

Khanna, S. (2008). Immunological and biochemical markers in oral carcinogenesis: The public health perspective. *International Journal of Environmental Research and Public Health. 5,* 418–422. 10.3390/ijerph5050418

Kumar S.S., Chhatwal R.J., Prasad A.K., & Mehta S.B. (2023). Isolated Phytochemicals and Biological Activities of *Dendrophthoe falcata*: A Review. *Asian Journal of Chemistry*. *35,* 4, 805-814. 10.14233/ajchem.2023.27582.

Lerma-Herrera, M. A., Beiza-Granados, L., Ochoa-Zarzosa, A., López-Meza, J. E., Navarro-Santos, P., Herrera-Bucio, R., Aviña-Verduzco, J., & García-Gutiérrez, H. A. (2022). Biological activities of organic extracts of the genus *Aristolochia*: A review from 2005 to 2021. *Molecules*, *27*(12), 3937.

Lerma-Herrera, M.A., Beiza-Granados, L., Ochoa-Zarosa, Lopez-Meza, J.E., Hernandez-Hernandez, J.D., Avina-Verduzco, J., & Garcia-Gutierrez, H.A. (2021). *In vitro* cytotoxic potential of extracts from *Aristolochia foetida* Kunth against MCF-7 and bMECs cell lines. *Saudi Journal of Biological Sciences*. *28,* 7082-7089. 10.1016/j.sjbs.2021.08.007

Mahomoodally, M.F. (2013). Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. *Evidence-Based Complementation Alternate Medicine. 5,* 20-28. 10.1155/2013/617459

Montiel-Ruiz, R.M., Córdova-de la Cruz, M., González-Cortázar, M., Zamilpa, A., Gómez-Rivera, A., López-Rodríguez, R., Lobato- García, C.E., & Blé-González, E.A. (2020). Antinociceptive effect of hinokinin and kaurenoic acid isolated from *Aristolochia odoratissima* L. *Molecules*. *25,* 1454. 10.3390/molecules25061454

Mosmann, T., (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65(1-2)*, 55-63. 10.1016/0022-1759(83)90303-4

Mukherjee, P.K., Kumar, V., Kumar, N.S., & Heinrich, M., (2008). The Ayurvedic medicine *Clitoria ternatea*—From traditional use to scientific assessment. *Journal of Ethnopharmacology, 120(3),* 291-301. 0.1016/j.jep.2008.09.009

Navarro-García, V. M., Luna-Herrera, J., Rojas-Bribiesca, M. G., Álvarez-Fitz, P., & Ríos, M. Y. (2011). Antibacterial activity of *Aristolochia brevipes* against multidrug-resistant Mycobacterium tuberculosis. *Molecules*, *16*(9), 7357-7364.

Nejhad, A.A., Behbahani, B.A., Hojjati, M., Vasiee, A., & Mehrnia, M.A. (2023). Identification of phytochemical, antioxidant, anticancer, and antimicrobial potential of *Calotropis procera* leaf aqueous extract. *Scientific Reports*. *13,* 14716. https://doi.org/10.1038/s41598-023-42086-1

Pattanayak, S.P., & Mazumder, P. (2011). Therapeutic potential of *Dendrophthoe falcata* (L.f.) Ettingsh on 7, 12-dimethylbenz (a) anthracene-induced mammary tumorigenesis in female rats: Effect on antioxidant system, lipid peroxidation, and hepatic marker enzymes. *Composite Clinical Pathology. 20(4),* 381–392. doi:10.1007/s00580-010- 1008-3

Rafe, M.R., Ahsan, M., Hasan, C.M., & Masud, M.M. (2015). Chemical and biological studies of leaf extract of *Dendrophthoe falcata* Linn. Dhaka University *Journal of Pharmaceutical Science*. *16,* 215. https://doi.org/10.3329/dujps.v16i2.35259

Rai, P.K., & Lalramnghinglova, H. (2010). Ethnomedicinal plant resources of Mizoram, India: Implication of traditional knowledge in health care system. *Ethnobotanical Leaflet. 6. https://opensiuc.lib.siu.edu/ebl/vol2010/iss3/6*

Rauf, A., Ibrahim, M., Alomar, T.S., AlMasoud, N., Khalil, A.A., Khan, M., Khalid, A., Jan, M.S., Formanowicz, D. & Quradha, M.M., (2024). Hypoglycemic, anti-inflammatory, and neuroprotective potentials of crude methanolic extract from *Acacia nilotica* L.–results of an *in vitro* study. *Food Science & Nutrition, 12(5)*, 3483-3491. https://doi.org/10.1002/fsn3.4017

Revathi, S., Govindarajan, R.K., Rameshkumar, N., Faruck, L.H., AlBuloshi M., Krishnan, M., & Kayalvizhi, N. (2017). Anti-cancer, anti-microbial and anti-oxidant properties of *Acacia nilotica* and their chemical profiling. *Biocatalysis and Agricultural Biotechnology. 11,* 322-329. https://doi.org/10.1016/j.bcab.2017.08.005

Rufai, Y., Isah, Y. & Isyaka, M.S., (2016). Comparative phyto-constituents analysis from the root bark and root core extractives of *Cassia ferruginea* (schrad D. C) plant. *Scholars Journal of Agriculture and Veterinary Sciences, 3(4),* 275-283. 10.21276/sjavs.2016.3.4.1

Sadiq, M.B., Tarning, J., Aye Cho, T.Z. & Anal, A.K., (2017). Antibacterial activities and possible modes of action of *Acacia nilotica* (L.) Del. against multidrug-resistant *Escherichia coli* and *Salmonella*. *Molecules, 22(1),* 47. 10.3390/molecules22010047

Salomé, D.C., Cordeiro, N., Valério, T.S., Santos, D.; Alves, P.B., Alviano, C.S., Moreno, D.S.A., & Fernandes, P.D. (2020). *Aristolochia trilobata*: Identification of the anti-inflammatory and antinociceptive effects. *Biomedicines*. *8,* 111. 10.3390/biomedicines8050111

Sastry, A.S., & Bhat, S., (2018). Essentials of medical microbiology. JP Medical Ltd. pp. 122

Sofowora, A.. Ogunbodede, E., & Onayade. A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional Complementary Alternate Medicines*. *10(5)*, 210–229. 0.4314/ajtcam.v10i5.2

Subramaniyan, V., Saravanan, R., Baskaran, D., & Ramalalingam, S. (2015). *In vitro* free radical scavenging and anticancer potential of *Aristolochia indica* L. against MCF-7 cell line. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(6), 392-396.

Tarfaoui, K., Brhadda, N., Ziri, R., Oubihi, A., Imtara, H., Haida, S., Al kamaly, O.M., Saleh, A., Parvez, M.K., & Fettach, S. (2022). Chemical Profile, Antibacterial and Antioxidant Potential of *Zingiber Officinale* Roscoe and *Elettaria cardamomum* (L.) Maton Essential Oils and Extracts. Plants. 11, 1487. https://doi.org/10.3390/plants11111487

Teoh, E.S. (2016). Medicinal Orchids of Asia; Springer: Berlin/Heidelberg, Germany. First edition. Springer. Kindle Edition.

Thara, N.K., & Raghavendra, M.P. (2022). Biologically active Naneoicglycolate of *Aristolochia littoralis* Parodi seed extract with anti-bacterial activity induces cytotoxicity and apoptosis in A431 human skin cancer cell line. *Indian Journal of Natural Products and Resources, 13(3)*, 301-309. 10.56042/ijnpr.v13i3.53169

Van Meerloo, J., Kaspers, G.J., & Cloos, J. (2011). Cell sensitivity assays: the MTT assay. *Cancer cell culture: methods and protocols,* pp.237-245. 10.1007/978-1-61779-080-5_20

Verma, P.K., Kewat, A.K., Rawat, K.K., & Anup Chandra, A.C. (2018). Invasion of *Aristolochia littoralis* Parodi in Kailash Sacred Landscape, Uttarakhand. *Indian Journal of Forestry*, *41(3)*, 231-233.

Wang, H., Hou, X., Li, B., Yang, Y., Li, Q., & Si, Y. (2021). Study on active components of *Cuscuta chinensis* promoting neural stem cells proliferation: bioassay-Guided Fractionation. *Molecules*. *26,* 6634.

10.3390/molecules26216634

Wang, H., Khor, T.O., Shu, L., Su, Z.-Y., Fuentes, F., Lee, J.-H., & Kong, A.-N.T. (2012). Plants vs. Cancer: A review on natural phytochemicals in preventing and treating cancers and their druggability. *Anticancer. Agents Medical Chemistry, 12*, 1281–1305. 0.2174/187152012803833026

Wang, L., & Weller, C.L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology, 17(6),* 300-312. https://doi.org/10.1016/j.tifs.2005.12.004

Yang, L., Chen, Q., Wang, F., & Zhang, G. (2011). Antiosteoporotic compounds from seeds of *Cuscuta chinensis*. *Journal of Ethnopharmacology. 135(2)*, 553-60. 10.1016/j.jep.2011.03.056

Zhang, Q.W., Lin, L.G. & Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine, 13,* 1-26. 10.1186/s13020-018-0177-x