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PHARMACOLOGICAL STUDIES IN *LUISIA ZEYLANICA* LINDL.

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Abstract

The present study deals with antimicrobial activity and anticancerous efficacy of leaf extract of *Luisia zeylanica* Lindl. by using different solvents. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, tannins, steroids, and terpenoids. These extracts were used to study antimicrobial activity against four bacterial and three fungal strains by agar diffusion method. *In vitro* anticancerous activity was also carried out against two cancer cell lines (MCF-7 and HeLa cell line) by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The highest zone of inhibition was observed in ethyl acetate extract against *Lactobacillus acidophilus,* whereas methanolic extract showed highest activity against fungus, *Candida albicans*. A significant cancer cell growth inhibition was observed for two extracts with IC_{50} value ranging between 18.36 μgml^{-1} and 67.914 μgml^{-1}.

Introduction

IN DEVELOPING countries, infectious diseases are major threat to public health. In India, a large number of people still rely on ethnomedicine to treat serious diseases including cancer and different types of inflammations. Contrary to synthetic drugs, ethnomedicinal plants are used to cure various infectious diseases. Many drugs from plant origin have no side effects and have enormous therapeutic potential to treat infectious diseases (Iwu *et al*., 1999). World Health Organization (WHO) has also recognized the importance of ethnomedicine/ traditional medicine in the healthcare sector. Recent ethnomedicinal and pharmacological studies on orchids indicate that these plants have immense potential for treatment of various diseases such as neurodegenerative disorders, and also as anticonvulsive, anticancer, antidiabetic *etc*. (Gntierrez, 2010; Shanavaskhan *et al*., 2012). Orchids are of ethnobotanical interest linking aboriginal man with plants for medicine (Paul and Hegde, 2001). Numerous orchid species possessing cultural values have been used in herbal medicines and also as food supplements by the tribal people across the world (Huda *et al.,* 2017; Joseph *et al.,* 2018; Khasim and Rao, 1999; Pathak *et al.,* 2010). Though there has been tremendous progress in medicinal plant research, orchids have not been utilized fully for their medicinal application. Orchid extracts and purified compounds are shown to exhibit several bioactivities such as antimicrobial, antioxidant, antihelmintic, insecticidal, antiviral, analgesic, antipyretic, and antiallergic activity.

Luisia zeylanica Lindl., a widely distributed epiphytic orchid found in Eastern Ghats of Visakhapatnam district shows multifarious ethnomedicinal properties against abscesses, chronic boils, burns, fractures, and tumours (Hossain, 2009). The knowledge of the chemical constituents of medicinal plants would further be important in understanding the actual value and mode of action of any ethnomedicine. Hence, scientific validation of ethnomedicinal plants provides evidence-based alternative medicines, which form the basis of discovery of new drugs. Antioxidant and phytochemical analysis of *L. zeylanica* leaf extracts has earlier been done by Sohag *et al.* (2017) and the present attempt was made to reveal its antimicrobial and anticancerous activities by using various solvent extracts using leaf material.

Material and Methods

During present study, some plants of *Luisia zeylanica* were collected from Paderu, Visakhapatnam District, Andhra Pradesh (voucher number ANUBH01210, Department of Botany and Microbiology, Acharya Nagarjuna University, Andhra Pradesh). Fresh healthy leaves were collected and washed thoroughly with distilled water to remove dust particles and shade dried at room temperature for ten days. The dried material was made into a coarse powder by means of electrical grinder. The dried powdered leaf material (200 g) was Soxhlet extracted with ethyl acetate and methanol solvents for about 12-15 hr. The crude extracts were evaporated by a vacuum rotary evaporator under reduced pressure. Various solvent extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4^0C until use.

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Table 1. Preliminary phytochemical screening of leaf extracts of *L. zeylanica.*

Phytochemicals	Test	Extracts		
		Hexane	Ethyl acetate	Methanol
Alkaloids	Dragendorff's test	-	-	-
Coumarins	Sodium hydroxide test	-	+	+
Flavonoids	Ferric chloride test	-	+	+
Glycosides	Anthrone test	-	+	-
Phenolic compounds	Phenol test	-	+	+
Quinones	H_2SO_4 test	-	-	-
Resins	Acetone H_2O test	-	-	+
Saponins	Foam test	-	-	+
Tannins	Braemer's test	-	-	+
Steroids	Salkowski test	-	+	-
Terpenoids	Salkowski test	-	+	-

(+), Positive (present); (-), Negative (absent).

Preliminary Phytochemical Screening

The leaf solvent extracts of *L. zeylanica* were preliminarily screened for the presence of phytochemicals by adopting standard procedures (Harborne, 1984).

Antimicrobial Study

The antibacterial activity of the crude extracts was determined by using both gram positive and gram negative bacteria. Three gram positive bacteria namely *Bacillus megaterium, Lactobacillus acidophilus,* and *Enterococcus faecalis* and three gram negative bacteria *Klebsiella pneumoniae, Proteus vulgaris,* and *Escherichia coli* were selected for this study. For antifungal activity, *Candida albicans* and *Aspergillus flavus* were used.

Nutrient agar (NA) and Czepak dox agar (Thom and Church, 1926) medium were used for the test of bacteria and fungi. Both nutrient media (100 ml) were sterilized at 15 lbs pressure (121°C) for 15 min, cooled and inoculated with 0.1 ml of bacterial and fungal test suspension. Now the mixed nutrient medium was poured into petri plates under aseptic conditions and allowed for solidification. Four wells of about 5 mm diameter were punched with a sterilized cork borer. Different concentrations of solvent extracts (50 µl, 100 µl, and 150 µl) were added to each well, and the addition of solvent alone served as control. The inoculated bacterial plates were incubated at 37^{0}C for 24 hr and fungal plates were incubated at 28^{0}C for 48 hr. The diameter of the inhibition zone was measured in millimetres.

Anticancerous Activity by MTT Assay

The two solvent extracts (ethyl acetate and methanol) were taken for *in vitro* cytotoxicity of MCF-7 and HeLa cell lines using MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Diluted leaf extract of 100 ml was added to 100 ml of media followed by the addition of cell lines (6×10^5) into 96 well micro-titer plate and incubated overnight at 37^{0}C for 48 hr. MTT was added

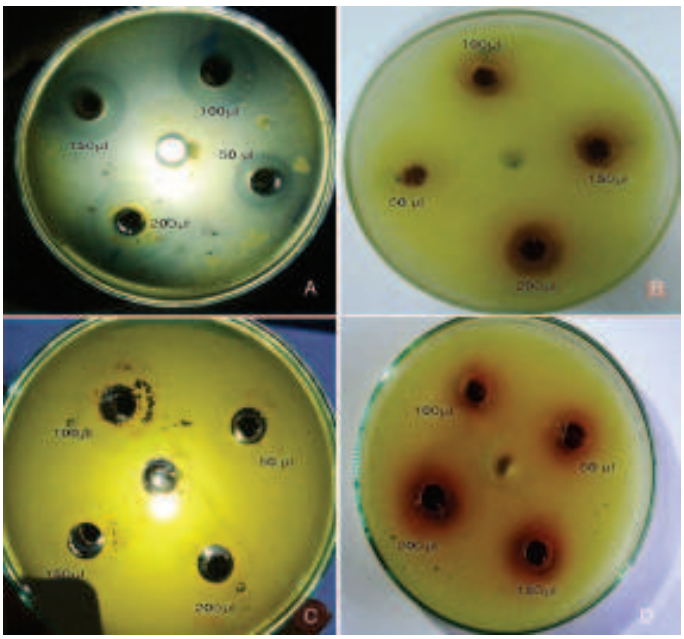

Fig. 1. A-D. Antimicrobial activity of ethyl acetate and methanolic extracts of *L. zeylanica* on *Lactobacillus acidophilus* (A and C), on *Candida albicans* (B and D**).**

Table 2. Antimicrobial study showing zone of inhibition of solvent extracts of *L. zeylanica.*

Microorganism	Zone of inhibition (mm)							
	Ethyl acetate extract				Methanol extract			
	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl
Bacillus megaterium	7±0.12	10±0.15	12±0.05	14±0.11	-	4±0.09	5±0.11	7±0.07
Lactobacillus acidophilus	7±0.14	9±0.09	13±0.13	18±0.12	-	3±0.07	7±0.14	9±0.11
Klebisiella pneumoniae	5±0.09	7±0.11	8±0.14	1±0.07	3±0.09	4±0.14	6±0.12	7±0.07
Escherichia coli	6±0.11	8±0.07	8±0.09	11±0.13	-	4±0.12	5±0.19	6±0.09
Enterococcus faecalis	5±0.13	8±0.13	11±0.10	13±0.07	3±0.11	4±0.09	6±0.12	7±0.11
Proteus vulgaris	6±0.15	9±0.12	10±0.09	17±0.15	-	2±0.11	4±0.09	5±0.07
Candida albicans	7±0.10	9±0.12	11±0.15	14±0.05	3±0.12	6±0.05	11±0.07	18±0.15
Aspergillus flavus	3±0.09	5±0.11	8±0.13	13±0.14	-	-	-	-
Penicillum citrinum	-	-	-	8±0.13	-	-	-	-

Entries in column nos. 2-9 are Mean ± S.D.

after the incubation, precipitates were formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density was measured at 570 nm on a microplate reader. Dose response curve used to calculate half maximal inhibitory concentration (IC_{50}) dose values (Bhatt, 2017).

Results and Discussion

Preliminary phytochemical screening of the different solvent extracts like ethyl acetate and methanol extract of leaves in *L. zeylanica* revealed the presence of various chemical compounds such as alkaloids, coumarins, flavonoids, glycosides, phenols, saponins, tannins,

Table 3. Cytotoxic properties of ethyl acetate extract of *L. zeylanica* on MCF-7 and HeLa cell lines.

Cell line	Concentration (μgml^{-1})	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (μgml^{-1})
MCF-7	100	0.821	0.823	0.825	0.823	0.816	39.65	
	75	0.915	0.917	0.918	0.916	0.909	44.169	
	50	1.043	1.045	1.047	1.045	1.038	50.437	
	25	1.098	1.101	1.102	1.1	1.093	53.109	48.439
	10	1.189	1.191	1.193	1.191	1.184	57.531	
	5	1.245	1.247	1.249	1.247	1.24	60.252	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	100	0.851	0.853	0.855	0.853	0.848	44.444	
	75	0.935	0.936	0.938	0.936	0.931	48.794	
	50	0.995	0.997	0.999	0.997	0.992	51.991	
	25	1.079	1.081	1.083	1.081	1.076	56.394	67.914
	10	1.186	1.188	1.189	1.187	1.182	61.949	
	5	1.272	1.274	1.276	1.274	1.269	66.509	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		

Table 4. Cytotoxic properties of methanolic leaf extract of *L. zeylanica* on MCF-7 and HeLa cell lines.

Cell line	Concentration (μgml^{-1})	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (μgml^{-1})
MCF-7	100	0.754	0.756	0.758	0.756	0.749	36.394	
	75	0.812	0.814	0.816	0.814	0.807	39.212	
	50	0.885	0.887	0.889	0.887	0.88	42.76	
	25	0.975	0.977	0.978	0.976	0.969	47.084	18.360
	10	1.062	1.064	1.066	1.064	1.057	51.36	
	5	1.133	1.135	1.137	1.135	1.128	54.81	
	Untreated	2.065	2.066	2.065	2.065	2.058	100	
	Blank	0.007	0.008	0.007	0.007	0		
HeLa	75	0.842	0.844	0.845	0.843	0.838	43.92	
	50	0.953	0.955	0.957	0.955	0.95	49.79	
	25	1.025	1.027	1.028	1.026	1.021	53.511	
	10	1.096	1.098	1.099	1.097	1.092	57.232	49.497
	5	1.295	1.297	1.299	1.297	1.292	67.714	
	Untreated	1.913	1.914	1.913	1.913	1.908	100	
	Blank	0.005	0.006	0.005	0.005	0		

steroids, and terpenoids (Table 1). Such secondary metabolites may be responsible for therapeutic properties of medicinal plants (Stray, 1998). Earlier investigations (Divya *et al*., 2012; Sheel *et al*., 2014)

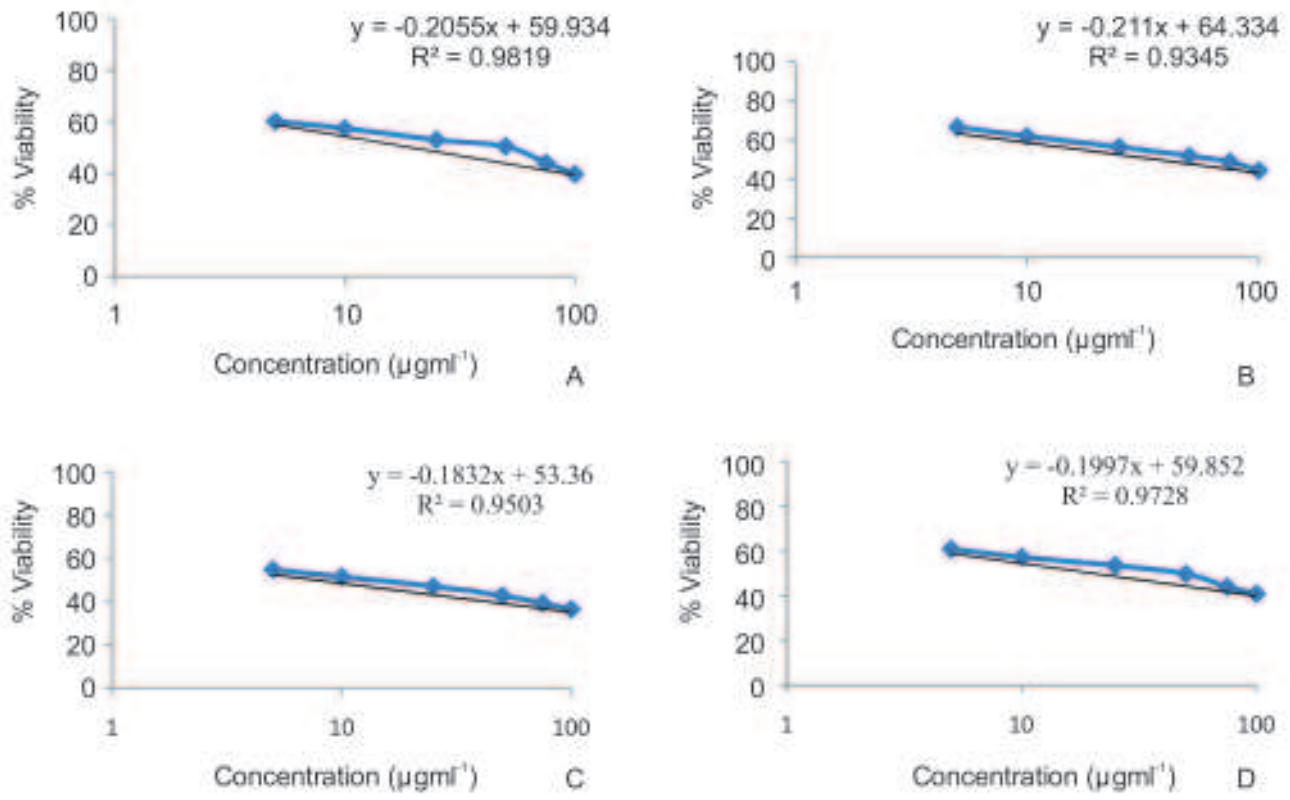

Fig. 2. A, Cytotoxic effect of ethyl acetate extract of *L. zeylanica* against MCF-7 cell lines; B, Cytotoxic effect of ethyl acetate extract of *L. zeylanica* against HeLa cell lines; C, Cytotoxic effect of methanolic extract of *L. zeylanica* against MCF-7 cell lines; D, Cytotoxic effect of methanolic extract of *L. zeylanica* against HeLa cell lines.

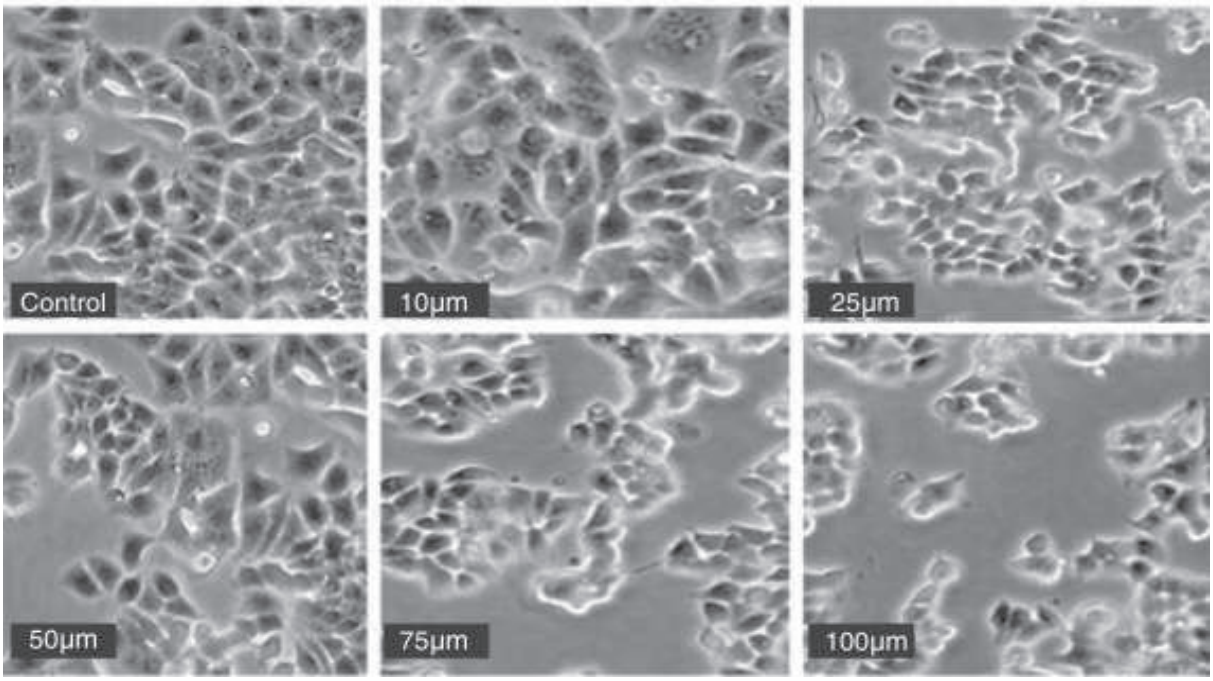

Fig. 3. Anticancerous activity of ethyl acetate extract of *L. zeylanica* on HeLa cell line.

also suggested the role of phytochemicals such as flavonoids, phenols, terpenoids, saponins, and tannins in antimicrobial and biological activities of various plant species.

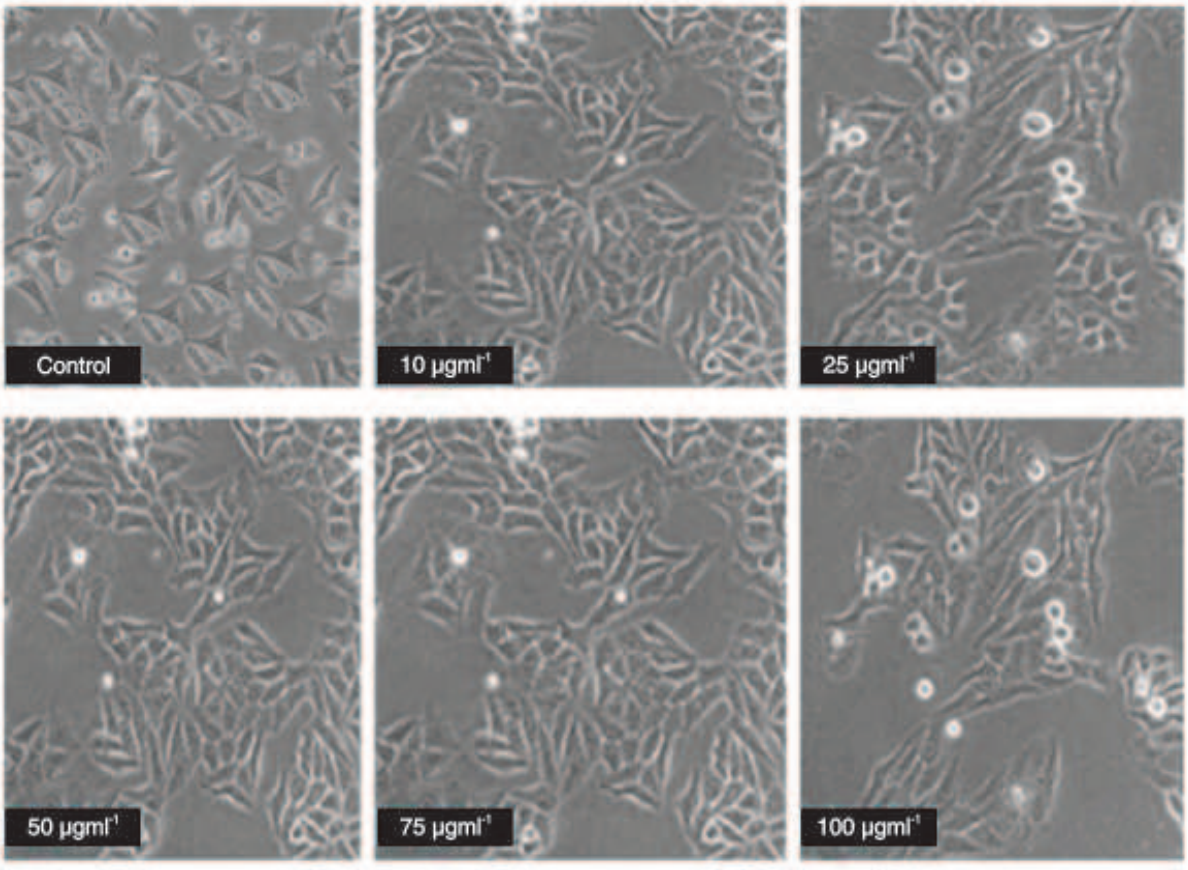

Fig. 4. Anticancerous activity of ethyl acetate extract of *L. zeylanica* on MCF cell line.

Antimicrobial Activity

Presently, ethyl acetate and methanolic crude extracts were taken for antimicrobial activity. The results are summarized in Table 2. The highest zone of inhibition was observed in ethyl acetate extract (200 µl) against *Lactobacillus acidophilus* (18 mm). Methanolic extract (200 µl) showed highest zone of inhibition (18 mm) against *Candida albicans* (Fig. 1. A-D). The degree of solubility of orchid phytoconstituents differs for various solvents (Manilal and Kumar, 1986). In a study on antimicrobial activity of *Luisia zeylanica* by Rashmi *et al*. (2015), the plant extract was observed to inhibit both gram positive and gram negative bacteria. However, in the present investigation, both extracts inhibited gram positive bacteria (*L. acidophilus*) more effectively when compared with gram negative bacteria. Triacontane reported in ethyl acetate extract of *L. zeylanica* (Jhansi, 2019) had antibacterial property as stated by Casuga *et al.,* (2016). Many compounds in methanolic extract have been reported to possess antimicrobial activity, such as phthalic acid-butyl hexyl ester and octadecenoic acid ethyl ester (Sudharshan *et al.,* 2010).

Anticancerous Activity

Medicinal orchids have significant role in prevention of cancer and its treatment (Bhatt *et al.,* 2018; Prasad and Koch, 2014, 2016). The IC_{50} value of less than 1000 μgml^{-1} for crude plant extract is toxic, while non-toxic (inactive) if it is greater than 1000 μgml^{-1} (Meyer *et al.,* 1982). In our study, death rate of MCF and HeLa cell lines increase with a rise in concentration of *L. zeylanica* leaf extract (Fig. 2. A-D). Anticancer activity of ethyl acetate and methanol leaf extract on MCF-7 and HeLa cell lines were shown in Figs. 3-6. The viability percentage of MCF-7 cell line of ethyl acetate and methanol leaf extracts at concentration 100 µg/ml reduced from 100% to 39.65% and 36.39%, respectively (Table 3). Similarly, for HeLa cell lines, it was reduced to 44.44% and 40.93% (Table 4). Results indicate that the methanolic leaf extract against MCF-7 cell line suppresses the cell proliferation and it showed good cytotoxicity when compared to HeLa cell lines. The lowest IC_{50} value (18.36 μgml^{-1}) was observed for

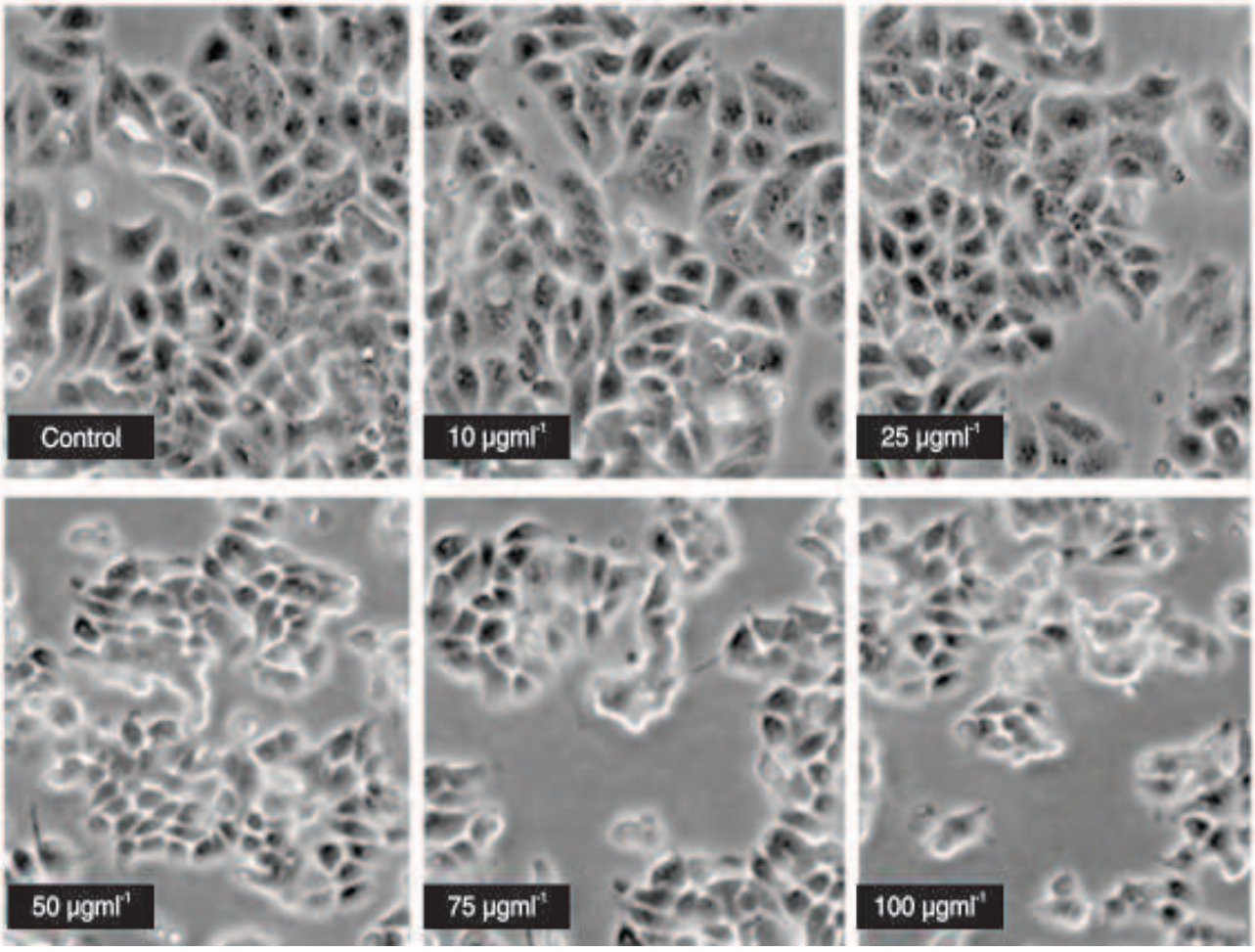

Fig. 5. Anticancerous activity of methanol extract of *L. zeylanica* on HeLa cell line.

methanolic leaf extract on MCF-7 cell lines. It indicates the inhibitory effect of methanolic leaf extract of *L. zeylanica* on breast cancer lines at different concentrations and represent *L. zeylanica* as potential chemotherapeutic agents to induce apoptosis in cancer cells. The present results were supported by previous anticancerous studies on orchids (Haridas *et al.,* 2016; Sohag *et al.,* 2017). Hence, the present findings proved that leaf extract of *L. zeylanica* have anticancer effect and this species could be employed to develop anticancer drugs.

The chemical compound, Kaempferol 3-glucoside present in the methanolic extract of *L. zeylanica* was reported to have antioxidant property (Kotani *et al.,* 2000). Antioxidants are molecules that can delay or prevent an oxidative reaction (Vilioglu *et al*., 1998) catalysed by free radicals, which is mainly due to the presence of phenolic compounds such as flavonoids and phenolic acids (Pietta, 1998). Octadecanoic acid, ethyl ester present in ethyl acetate extract was shown to have anticancer property (Aydin *et al*., 2005). Sohag *et al*. (2017) reported that the methanolic fraction of *L. zeylanica* leaf possesses scavenging activity. In the present study also, methanolic extract was effective in inhibiting MCF-7 cell lines with highest *in vitro* cytotoxicity and least IC_{50} value (18.36 µg/ml) reflecting its antioxidant and scavenging activity. Other phenolic compounds such as tannins, coumarins, and quinones were reported from this species and Jhansi (2019) had shown its antioxidant properties that could help in combating various diseases. According to Mahabub *et al*. (2013), phenolic compounds may be therapeutic agents for the treatment of leukemia by causing decreased cell viability and inducing apoptosis, and these are associated with reduced risk of liver (Stagos *et al*., 2012), lung (Lee *et al*., 2011), and cervical cancers (Araujo *et al.,* 2011); these compounds are known to regulate cell proliferation, survival, and apoptosis.

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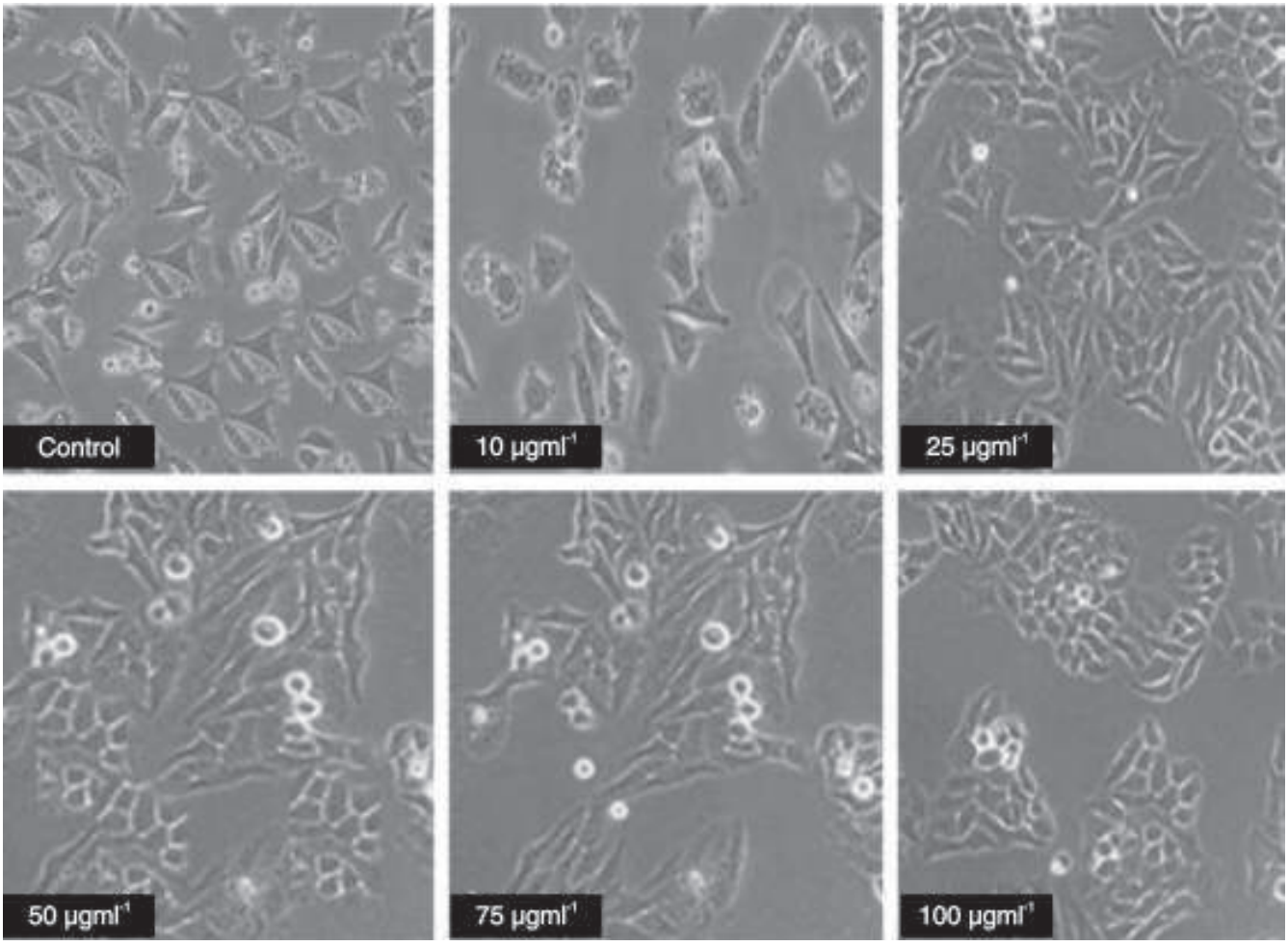

Fig. 6. Anticancerous activity of methanol extract of *L. zeylanica* on MCF cell line.

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References

Araujo, J. R., P. Gonzalves, and F. Martel. 2011. Chemoprotective effect of dietary polyphenols in colerectal cancer cell lines. *Nutr. Res.,* **31**: 77-87.

Aydin, S., I. H. Ozercan, F. Dogli, S. Aydin, O. Dogur, S. Celebi, O. Akin, and S. P. Guzel. 2005. Ghrelin immunohistochemistry of gastric adenocarcinoma and mucoepidermoid carcinoma of Salivary gland. *Biotech. Histochem.,* **80**: 160-68.

Bhatt, R. P. 2017. Anticancer activities of plant extracts of *Gymnacranthera farquhariana* (Hook. f. & Thomson) Warb., *Myristica fatua* Houtt. var. *magnifica (*Beddome) Sinclair and *Samadera indica* Gaertner. *Adv. Obes. Weight Manag. Control*, **6**: 167-71.

Bhatt, D. R., K. D. Jethva, and M. N. Zaveri. 2018*. In vitro* cytotoxicity studies of the therapeutic orchid: *Eulophia nuda*. *J. Pharmacogn. Phytochem.,* **7**(4): 680-83.

Casuga, F. P., A. L. Castillo, and M. J. A. T. Corpoz. 2016. GC-MS analysis of bioactive compounds present in different extracts of an endemic plant *Broussonetia luzonica* (Moraceae) leaves. *Asian Pac. J. Trop. Biomed.,* **6**: 957-61.

Divya, K., H. R. Pradeep, K. K. Kumar, K. R. H. Venkatesh, and T. Jyothi. 2012. Herbal drug *Swietenia mahogany* Jacq- A review. *Glob. J. Res. Med. Plants Indig. Med.,* **1**: 557.

Gntierrez, R. M. P. 2010. Orchids- A review of uses in traditional medicine, its phytochemistry and pharmacology. *J. Med. Plants Res.,* **4(**8): 592-638.

Haridas, R., S. Manorama, and S. Thekkan. 2016. *In vitro* cytotoxicity activity of *Malaxis rheedii* Sw. methanol extract against hela cell line and mcf-7 cell line. *Asian J. Pharm. Clin. Res*., **9** (6): 244-46.

Horborne, J. B. 1984. *Phytochemical Methods*. Chapman and Hall publications, London, U.K.

Hossain, M. M. 2009. Traditional therapeutic uses of some indigenous orchids of Bangladesh. *Med. Aromat. Plant Sci. Biotechnol.*, **3**: 100-06.

Huda, M. K., A. Price, and C. C. Wilcock. 2017. Identification of medicinal orchids of Bangladesh: DNA barcoding vs. traditional taxonomy. *J. Orchid Soc. India*., **31**: 33-40.

Iwu, M. W., A. R. Duncan, and C. O. Okunji. 1999. New antimicrobials of plant origin. *In: Perspectives on New Crops and New Uses*, (ed. J. Janick) pp. 457-62. ASHS Press, Alexandria, Va, U.S.A.

Jhansi, K. 2019. *Ethnobotanical and Phytochemical Studies with Special Reference to Therapeutic Properties of Four Orchid Species from Eastern Ghats of India.* Ph.D. Thesis, Acharya Nagarjuna University, Nagarjuna Nagar, India.

Joseph, M., L. Jose, and S. Sequeira. 2018. A comparative phytochemical screening of four epidendroid orchids of Kerala, India. *J. Orchid Soc. India*., **32**: 41-43.

Khasim, S. M. and P. R. M. Rao. 1999. Medicinal importance of orchids. *The Botanica*, **49**: 86-91.

Kotani, M., M. Matsumoto, H. S. Fujita, W. Wang, M. Suemera, T. Kishimoto, and T. Tanaka. 2000. Persimon leaf extract and astragalin inhibit development of dermatitis and IgE elevation in Nc/Nga mice*. J. Allergy Clin. Immunol.,* **106** (1): 159-66.

Lee, H., C. Kang, E. Jung, J. S. Kim, and E. Kim. 2011. Anti-metastatic activity of polyphenol-rich extract of Eeklonia cava through the inhibition of the Akt pattwar in A549 human lung cancer. *Food Chem*., **127(**3): 1229-36.

Mahbub, A. A., C. L. Maitre, S. Haywood-Small, G. J. McDougall, N. A. Cross, and N. Jordan-Mahy. 2013. Differential effects of polyphenols on proliferation and apoptosis in human myeloid and lymphoid leukemia cell lines. *Anti-Cancer Agents ME (Formerly Curr. Med. Chem.-Anti-Cancer Agents)*, **13**(10): 1601-13.

Manilal, K. S. and C. Sathish Kumar. 1986. Researches on Indian Orchids. *In: Biology, Conservation and Culture of Orchids* (ed. S. P. Vij). East-West Press, New Delhi, India.

Meyer, B., N. Ferrigni, J. Putnam, L. Jacobsen, D. J. Nichols, and J. L. McLaughlin. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.,* **45**(5): 31-34.

Pathak, Promila, A. Bhattacharya, S. P. Vij, K. C. Mahant, Mandeep K. Dhillon, and H. Piri. 2010. An update on the medicinal orchids of Himachal Pradesh with brief notes on their habit, distribution and flowering period. *J. Non Timb. For. Prod.*, **17**(3): 365-72.

Paul, S. and S. N. Hegde. 2001. Some orchids of ethnobotanical interest. *In: Orchids: Conservation, Culture, Farming and Trade* (ed. S. N. Hegde). Himalayan Publishers, New Delhi, India.

Pietta, P. G. 1998. Flavonoids in medicinal plants. *In: Flavonoids in the Health and Disease* (eds. C. A. Rice-Evans and L. Packer) pp. 61-110. Dekker, New York, U.S.A.

Prasad, R. and B. Koch. 2014. Antitumor activity of ethanolic extract of *Dendrobium formosum* in T-Cell Lymphoma*:* An *in vitro* and *in vivo* Study. *Biomed. Res. Inter.*, Article ID 753451.

Prasad, R. and B. Koch. 2016. *In vitro* anticancer activities of ethanolic extracts of *Dendrobium crepidatum* and *Dendrobium chrysanthum* against T-cell lymphoma, *J. Cytol. Histol*., **7**: 1-8.

Rashmi, K., S. D. Shweta, C. S. Sudeshna, P. S. Vrushala, T. P. Kekuda, and H. L. Raghavendra. 2015. Antibacterial and radical scavenging activity of selected orchids of Karnataka, India. *Sci. Tech. Arts Res. J.*, **4**(1): 160-64.

Shanavaskhan, A. E., M. Sivadasan, A. H. Alfarhan, and J. Thomas. 2012. Ethnomedicinal aspects of angiospermic epiphytes and parasites of Kerala, India. *Ind. J. Tradit. Know.,* **11**(2): 250-58.

Sheel, R., K. Nisha, and J. Kumar. 2014. Preliminary phytochemical screening of methanlic extract of *Clerodendron infortunatum*. *ISRO J. Appl. Chem.,* **7**: 10-13.

Sohag, S. I., M. M. Hoque, and M. K. Huda. 2017. Phytochemical screening and antioxidant activity of rare medicinal orchid,

Luisia zeylanica Lindl. *J. Pharmacogn. Phytochem.*, **6**(4): 688-92.

Stagos, D., G. D. Amoutzias, A. Matakos, A. Spyrou, A. M. Tsatsakis, and D. Kouretas. 2012. Chemoprevention of liver cancer by plant polyphenols. *Food Chem. Toxico.*, **50**(6): 2155-70.

Stray, F. 1998. *The Natural Guide to Medicinal Herb and Plants*. Tiger Books International, London, U.K.

Sudharsan, S., A. Saravanan, A. Shanmugam, S. Vairamani, R. Mohan Kumar, S. Menaga, and N. Ramesh. 2010. Isolation and characterization of octadecanoic acid from the ethyl acetate root extract of *Trigonella foneum-graecum* L. by using hydroponics method. *J. Bioterr. Biodef.*, **2:**105. Doi: 10.4172/2157-2526.1000105.

Thom, C. and M. Church. 1926. *The Aspergilli.* Williams and Wilkins comp. Baltimore, U.S.A.

Velioglu, Y. S., G. Mazza, L. Cao, and B. D. Oomah.1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.,* **46**: 4113-17.