

## In Vitro Evaluation of Anticancer and Antimicrobial Activity of Selected Medicinal Plants for Breast Cancer



### Biotechnology

**KEYWORDS :** S. cumini, C. igneus, M. pruriens, Anitdiabetic,  $\alpha$ - Glucosidase, aldose reductase.

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### ABSTRACT

*The use of natural substances to inhibit carcinogenesis is a rapidly evolving aspect of cancer research. In present investigation the methanolic extract of Taxus baccata, lallemantia royleana and Podophyllum hexandrum were evaluated for their in vitro anticancer and antimicrobial activity. The results obtained indicates that Podophyllum hexandrum possess a potential inhibiting activity of IC<sub>50</sub> 116 $\mu$ g/mL towards MCF-7. The selected plant samples were also assessed for their antimicrobial activity against Bacillus subtilis and Aspergillus niger and the minimum inhibitory concentrations (MICs) were determined using micro dilution assay. In general, it was observed that the extract of Podophyllum hexandrum was found to be more effective against selected microbial strains. The results of the present findings may be useful for the discovery of novel anticancer and antimicrobial agents from the plant origin.*

### INTRODUCTION

Cancer is a major class of disease in both developed and developing countries, occurs due to some changes in the DNA sequence within the cell. The causes of cancer are diverse, complex and only partially understood, some of them are physical, environmental, metabolic, chemical and genetic factors, which play a direct or indirect role in the stimulation of cancers [1]. About 10 million new cases are diagnosed and over 6 million deaths occur worldwide annually because of cancer [2]. The risk of developing cancer generally increases with age [3]. The chances of surviving the disease vary greatly by the type, location of the cancer and the stages of disease. Chemotherapy is a plant derived phytochemicals has appeared as an accessible and promising approach to cancer control and management [4]. Because of its high death rate and serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and complementary methods of treatment. Recent researches revolve round the urgency to evolve suitable chemotherapy consistent with new discovery in cell biology for the treatment of cancer with no toxic effect on the healthy cells.

At Present days various therapies are available for the treatment of cancer such as, chemotherapy, radiotherapy etc. Besides these expensive therapies phytotherapy plays a significant role for the treatment of the cancer. Since medieval times, plants are the main source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain even in the 21st century an integral part of the health care in different countries, especially the developing ones. In the late 90's, the WHO stated that a big percentage of the world's population depends on plant based therapies to cover the needs of the primary health care (WHO 1999) [5].

### MATERIALS AND METHOD

#### Plant Materials

Healthy plant parts of three medicinal plants *Taxus baccata*, *lallemantia royleana* and *Podophyllum hexandrum* were collected in and around Bangalore, Karnataka state. The plant material were Washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter and used for preparation of extract [6].

#### Solvent Extraction:

The air-dried and powdered plant materials (20g of each) were extracted with 250 ml methanol (CH<sub>3</sub>OH) by Soxhlet apparatus 60°C, 8hrs [7-8]. The crude extracts were filtered and the filtrate evaporated using a rotary evaporator. The dissolved constituents were further dried under pressurized vacuum conditions. Stock solutions were prepared by dissolving the dried

residue in Dimethylsulphoxide (DMSO). Extract solutions were stored at -20°C until use.

#### Phytochemical evaluation [9]

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the phytoconstituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1989).

#### (a) Test for reducing sugars

One gram of the aqueous extract was weighed and placed into a test tube. This was diluted using 10 ml of de-ionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40°C in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethylsulphoxide (DMSO) as the diluents for the ethanolic extract.

#### (b) Test for resins

Two grams of the ethanol extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material

#### (c) Test for tannins

Two grams of the aqueous extract was weighed and placed in a test tube. Two drops of 5% ferric chloride solution was then added. The appearance of a dark green colour was indicative of the presence of tannins. The same procedure was repeated using the ethanol extract.

#### (d) Test for steroid glycosides

One gram of the ethanol extract was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer were indicative of the presence of steroid glycosides. Same procedure was repeated using the aqueous extract.

#### (e) Test for Flavonoids

Two grams of the ethanol extract was weighed, placed in a test tube, followed by the addition of 10 ml of DMSO. The mixture was heated, followed by the addition of magnesium metal and 6 drops of concentrated hydrochloric acid. The appearance of red

colour was indicative of the presence of flavonoids. Same procedure was repeated using aqueous extract.

#### (f) Test for alkaloids

One gram each of the ethanol extract was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragendoff's reagent was added while 2-3 drops of Meyer's reagent were added to the second test tube. The development of an orange-red precipitate (turbidity) in the first test tube (with Dragendoff's reagent) or white precipitate (turbidity) in the second test tube (with Meyer's reagent) was indicative of the presence of alkaloids. Same procedure was repeated using aqueous extract

#### (g) Test for Saponins

Five grams of the aqueous extract was weighed and placed in a test tube. This was followed by the addition of 5 ml de-ionized distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins. Same procedure was repeated using DMSO for the ethanol extract

#### Antimicrobial susceptibility test

The investigated microorganisms consisted of *Bacillus subtilis* and *Aspergillus niger*. Microorganisms were obtained from the ATCC. Microorganisms were maintained at 4 °C on nutrient agar slants. The antimicrobial assay was performed by agar disc diffusion method [10]. The 20ml of sterilized Muller Hinton Agar was poured into sterile petri plates, after solidification, 100 µl of fresh bacterial culture were swabbed on the respective plates. Each of discs which are approximately 5mm in diameter was cut from Whatman filter paper. The sterile discs were kept over the agar plates using sterile forceps at various concentrations (2, 4, 6, 8, and 10µl). The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) recorded [11].

#### DPPH ASSAY

Antioxidant activity (DPPH free radical scavenging activity) of the extract was ranging from 100, 50, 25, 12.5; 6.25 microgram/ml was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method [12]. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given [13].

Percent (%) inhibition of DPPH activity = (A-B/A) 100

Where A = optical density of the blank and B = optical density of the sample.

#### Cell Culture

In this study we have used human MCF-7 cell line which was obtained from the American Tissue Culture Collection (ATCC). Culture conditions consists of a suitable vessel containing a medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (O<sub>2</sub>, CO<sub>2</sub>), and regulates the physicochemical environment (pH, osmotic pressure, temperature).

Cytotoxic activity (MTT assay)

The cytotoxic assay detects the reduction of MTT [3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase to blue insoluble formazan product, which reflects the normal functioning of mitochondria and hence the cell viability. Briefly 5.0 X 10 cells of MCF-7 were plated in triplicate in 96 well plates with RPMI-1640 and incubated for 24 hrs at 37 °C. Plant extracts were tested as 10, 20, 40, 80, 160 and 320 µg/ml in serum free RPMI media and incubated for 24 hr in CO<sub>2</sub> incubator at 37°C. After incubation with plant extracts, the media was removed from the wells and added 100 µl/well of the MTT reagent and incubated for 3-4 hrs. After incubation, the MTT reagent was removed before adding 100 µl DMSO to each well and gently shaken. Plant extracts treated cells were compared to untreated cell control wells. Measure the absorbance at 590nm using a Tecan microplate reader. The percentage inhibition was determined using the formula.

% Inhibition = 100-(optical density of sample/optical density of control) × 100.

IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell [14-17].

#### RESULTS AND DISCUSSIONS

Totally three crude methanolic extracts of *Taxus baccata*, *Lallemantia royleana* and *Podophyllum hexandrum*, were analysed by various qualitative phytochemical tests to determine the active constituents like Alkaloids, Saponins, Flavanoids, Phlobatanin, Tanins, Terpenoids, Glycosides, Steroids, Carbohydrates and Amino acids. Based on Phytochemical analysis provide an understanding of the biological activities which a particular plant will have as shown in Table 1.

Sl No	Phyto-constituents	TB1	B2	P3
1	Molish Test	-	+	+
2	Alkaloids	-	-	-
3	Tanins	+	-	-
4	Saponins	-	-	-
5	Phlobatanins	+	-	-
6	Flavanoids	-	-	+
7	Terpenoids	-	-	-
8	Glycosides	-	-	-
9	Steroids	-	+	-
10	Amino acids	-	-	-

**Table 1 showing phytochemical analysis of methanolic extract of *Taxus baccata*=TB1, *Lallemantia royleana*=B2 and *Podophyllum hexandrum*=P3.**

+ve = present, -ve = absent.

To determine MICs using agar disc diffusion method showed that plant extracts exhibited considerable antimicrobial effect i.e zone of inhibition against test microorganisms. The antimicrobial activity of plant extracts (10 mg/100 microlitres) gave different zones of inhibition on the bacterial species like *Bacillus subtilis*, *Aspergillus niger*.among these three only *Podophyllum hexandrum* showed antimicrobial activity shown in the figure 1.



Figure 1: The antimicrobial activity of methanolic plant extracts by Disc diffusion method.

The methanolic extract (20mg/ml) of both *Taxus baccata*, *Podophyllum hexandrum* are giving a better activity of 29.8742mg/ml, 35.8541mg/ml respectively. *Lallemantia royleana* didn't show a good antioxidant activity as show in table 2.

Sample	Conc (mg/ml)	Mean Absorbance	DPPH radical scavenging activity	IC50
<i>Taxus baccata</i>	0.000	1.663	0.000	29.8742
	6.25	0.688	58.752	
	12.5	0.6219	62.606	
	25	0.4962	70.184	
	50	0.3212	86.700	
	100	0.0681	95.905	
<i>Lallemantia royleana</i>	0.000	1.663	0.000	-
	6.25	0.8247	50.432	
	12.5	0.8211	50.628	
	25	0.8389	48.558	
	50	0.7099	57.313	
	100	0.7507	54.861	
<i>Podophyllum hexandrum</i>	0.000	1.663	0.000	35.8541
	6.25	0.8701	47.682	
	12.5	0.8434	49.608	
	25	0.7643	54.044	
	50	0.6328	60.748	
	100	0.3805	77.121	

Table 2: Percentage of Radical scavenging activity of *Taxus baccata*, *Lallemantia royleana* and *Podophyllum hexandrum*.

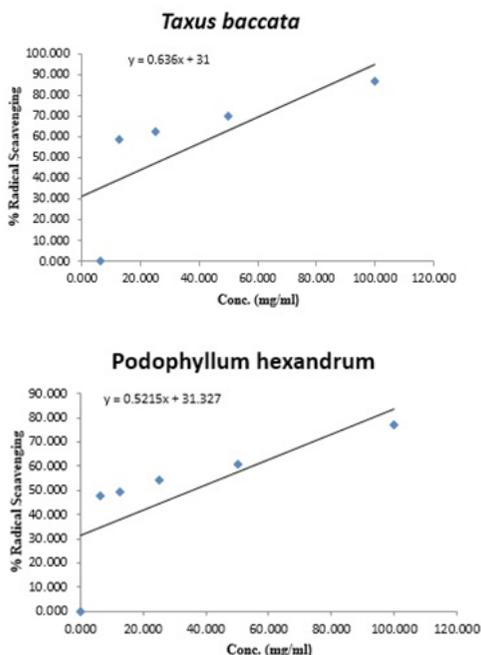


Fig: Percentage of radical scavenging of *Taxus baccata* and *Podophyllum hexandrum*.

To analyse the possible anti-proliferative effect of plant extracts as a first step toward the development of novel alleged anticancer agents, we tested methanol extract of *Taxus baccata*, *Lallemantia royleana* and *Podophyllum hexandrum*, on inhibition and proliferation to check for their capability to inhibit cell growth or viability on MCF-7 cell line at concentrations of 0, 10, 20, 40, 80, 160 and 320 µg/ml. Proliferation of these cells was significantly inhibited by above extracts in a concentration-dependent manner for 24 h, as shown in Table 1. The IC50 values of *Podophyllum hexandrum* is 116µg/ml.

Plants name	Conc. µg/ml	OD 590 nm	% Inhibition	IC50
<i>Taxus baccata</i>	1%DMSO	0.9365	0.00	-
	10	0.8852	5.48	
	20	0.8567	8.52	
	40	0.8531	8.91	
	80	0.7628	18.55	
	160	0.7345	21.57	
<i>Lallemantia royleana</i>	10	0.8375	10.57	-
	20	0.8284	11.54	
	40	0.7928	15.34	
	80	0.7883	15.82	
	160	0.7539	19.50	
	320	0.7714	17.63	
<i>Podophyllum hexandrum</i>	10	0.84576	9.69	116
	20	0.6974	25.53	
	40	0.5894	37.06	
	80	0.4731	49.48	
	160	0.2205	76.45	
	320	0.0564	93.98	

Table 3: Effect of selected plants on the cancer cell line MCF-7

In the present study, the ethanolic extracts of selected medicinal plants were evaluated for their anticancer and antimicrobial potential. Currently chemotherapy is regarded as one of the most efficient cancer treatment approach. Although chemotherapy significantly improves symptoms and the quality of life of patients with cancer, only modest increase in survival rate can be achieved. Faced with palliative care, many cancer patients use alternative medicines, including herbal therapies. Medicinal plants are well known for their immunomodulatory and antioxidant properties, leading to anticancer activities. They are known to have versatile immunomodulatory activity by stimulating both non specific and specific immunity [18-19]. Plants contain several phytochemicals, which possess strong antioxidant activities. The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by 'free radicals'. Many plant-derived products have been reported to exhibit potent antitumor activity against several rodent and human cancer cell lines [20]. Phytochemicals such as vitamins (A, C, E, and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to elicit anticancer activities. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer [19, 20]. Varieties of medicinal plants have been reported effective in various types of malignant (cancer) and benign tumors of humans and experimental animals.

CONCLUSION

The obtained data, totally the extract of above mentioned medicinal plants can be considered as a resource for potential anticancer and antimicrobial agents. Also, the selected plants can be further exploited for the discovery of novel anticancer/antimicrobial agents. Nevertheless, the present findings may also supplement and strengthen the process of standardization and validation of herbal drugs containing active ingredients derived from the selected medicinal plants.

## REFERENCE

1. ShahedurRahman, FaizusSalehin and Asifqbal: In vitro antioxidant and anticancer activity of young Zingiberofficinale against human breast carcinoma cell lines. *BMC Complement Alt Med.* 2011; 11:76. | 2. Parkin, D.M.; Bray, F.; Ferlay, J.; Pisani, P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer.* 2001;94:153–156. | 3. Gueritte F, Fahy J; The vinca alkaloids. In *Anticancer Agents from Natural Products*. Edited by Cragg GM, Kingston DGI, Newman DJ. Boca Raton, Florida. Taylor & Francis Group. 2005; 123–136. | 4. Surh TJ Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer.* 2003; 3:768-80. | 5. Naik VN. Flora of Marathwada (vol-I, II). Aurangabad (MS), India: Amrut Prakashan, 1998. | 6. Satish S, Raveesha KA, Janardhana GR. Antibacterial activity of plant extracts on Phytopathogenic *Xanthomonas campestris* pathovars. *Letters in Applied Microbiology.* 1999; 28 : 145-147. | 7. C Yompakdee, S Thunyaharn, and T Phaechamud. Bactericidal Activity of Methanol Extracts of Crabapple Mangrove Tree (*Sonneratiacaseolaris* Linn.) Against Multi-Drug Resistant Pathogens. *Indian J Pharm Sci.* 2012; 74(3): 230–236. | 8. Parimalakrishnan Sundararajan, Akalanka Dey, Anton Smith, Arul Gana Doss, Manavalan Rajappan and Sridhar Natarajan. Studies of anticancer and antipyretic activity of *Bidens pilosa* whole plant. *African Health Sci.* 2006; 6(1): 27-30. | 9. Akshay Kumar R, Nuthan Prasad B. C. & Dr. Lingayya Hiremath, Evaluation of phytochemical screening & antibacterial activity of aqueous, ethanol extracts of medicinal plants against few common microorganisms. *VCFL Sciences Journal*, Vol 2, Issue 4, Start page: 17-28; Date: 2012; | 10. Bauer A. W., Kirby W.M.M., Sherris J.C. Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol.* 45: 493-496, (1966). | 11. Nair R., Kalariya T., Chanda S., Antibacterial activity of some selected Indian medicinal flora, *Turk J Biol.* 29: 41-47, (2005). | 12. Braca A, Sortino C, Politi M. Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. *J Ethnopharmacol* 2002;79:379-381. | 13. Bors W, Saran M, Elstner EF. Screening for plant antioxidants. In: Linskens HF, Jackson JF, editors. *Modern Methods of Plant Analysis Plant Toxin Analysis* New Series, Vol 13. Springer, Berlin; 1992: p. 277-295 | 14. Ramzi A Mothana, Ulrike Lindequist, Renate Gruenert and Patrick J Bednarski. "Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqatra. *BMC Complement Alt Med.* 2009;9:7. | 15. DA Scudiero, RH Shoemaker, K. D. Paull et al., Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.* 1988; 48:4827–4833. | 16. Huzaimah Abdullah Sani MSc, Asmah Rahmat PhD, Maznah Ismail PhD. "Potential anticancer effect of red spinach (*Amaranthus gangeticus*) extract. *Asia Pac J Clin Nutr.* 2004; 13 (4):396-400. | 17. Aiello M D'Esposito, E Fattorusso et al. Novel bioactive bromopyrrole alkaloids from the Mediterranean sponge *Axinellaverrucosa*. *Bioorg Med Chem.* 2006; 14: 17–24. | 18. Agrawala SK, Chatterjee S, Misra SK. Immunopotentiating activity of a polyherbal formulation 'Immu-21' (research name). *Phytomedica*, 2001, 2: 1-22. | 19. Lin YL, Juan IM, Chen YL, Liang YC, Lin JK. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical absorbing capacity with anti-proliferative actions in fibroblast cells. *J Agric Food Chem.* 1996, 44: 1387-1394. | 20. Caragay AB. Cancer-preventative foods and ingredients. *Food Technol.* 1992, 46: 65-68. |