



Studies on Anticancer and Antibacterial Potentialities of *Garuga pinnata* roxb

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ABSTRACT

Methanolic extract of leaf, stem, fruit and stem bark of *Garuga pinnata* was studied for the evaluation of anticancer and antibacterial activities. The cytotoxic effects of the extracts was tested upon napulii larva's (*Artemia nauplii*) by conducting brine shrimp lethality test, which showed significant percentage death of larva's. Anticancer activities of the extracts were evaluated using MCF-7 and MDA-MB-231 human breast cancer cell lines. The results showed that stem bark possesses predominant activity on MCF-7 but not on MDA-MB-231 cell line and no other extracts exhibited anticancer properties on both the cell lines. The antibacterial activity was assayed by agar diffusion method using *Bacillus subtilis*, *B.cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi* and *Klebsiella pneumonia*. Among the tested organisms, *B.subtilis* and *pneumonia* exhibited high susceptibility towards leaf and stem bark extracts, compared to known standard, gentamycin.

Keywords: Cytotoxic, *Garuga pinnata*, Napulii larva.

INTRODUCTION

Majority of world population are hinged around medicinal plants as source for remedies to cure and prevent all kinds of human disorders.¹ Among 500,000 of plants species on earth, it is estimated that a relatively small percentage (1 to 10%) of these are used as food and medicinal source.²⁻³ The search for natural drugs has always been of great interest to the research community. In recent years, number of studies has been reported, dealing with natural drug development against multi-drug resistant microorganisms.⁴ Naturally derived drugs are already proven as very effective in preventing hazardous effects generated by the chemicals during the chemotherapeutic treatment of various cancers.⁵ These natural drugs have been attributed to their ability to target the mechanism of cancer cell division.⁶ The mechanism underlying their pharmacological activities is though not clear; it has been speculated for their potentiality to inhibit certain enzymes or quenching of free radical generation or modulation of steroid hormone concentrations etc. It has been reported that among 12,000 phenols which were isolated from various medicinal plants, only 10% are screened for their role as antimicrobial agents.⁷ The best examples of phytochemicals studied as antimicrobial agents are Phytoanticipins (saponin avenacin A-1 and saponin α -tomatine) and Phytoalexins (scopoletin, camalexin, nomilactone B) are isolated from tobacco, *A. Thaliana*, rice, *Brassicaceae*, major oat root and tomato.⁸

Garuga pinnata, (*Burseraceae*) commonly called as golika or kakad is one such medicinal plant possesses several pharmacological properties, where, few are already reported and few are yet to be investigated. The leaves of

this plant are found to be having noticeable amount of phenolic compounds, which may involve in controlling various oxidative and reductive processes.⁹⁻¹⁰ The fruits are stomachic and expectorant; given in diarrhea whereas, the stem juice is commonly used as eye drops to cure opacities of the conjunctiva.⁹⁻¹⁰ The stem bark of this plant in the combination of pepper is used to treat the diabetes.¹¹ In contrast to the above mentioned medicinal properties of this plant, the current investigation was carried out to evaluate anticancer and antibacterial activities of methanolic fractions of various parts of *Garuga pinnata*.

MATERIALS AND METHODS

Plant Material

Leaves, fruits, stem and stem bark of *Garuga pinnata* were collected from Rampet, Warangal District, Andhra Pradesh. The species has been authenticated by Prof. V.S Raju, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, India.

Extraction Procedure

Leaves, fruits, stem and stem bark of *Garuga pinnata* were chopped in to smaller fragments, dried under shade and grinded in homogenizer to coarse powder. The powder of each part (100 grams) was used for extraction with methanol and dried under rotary evaporator at 50°C.

Bacterial and Fungal Cultures

Strains of *Staphylococcus aureus* ATCC 96, *Bacillus subtilis* MTCC 441, *Bacillus cereus*, *Klebsiella pneumonia* MTCC 109, *Escherichia coli* ATCC 8739, *Salmonella typhi* ATCC 4420 and *Bacillus cereus* ATCC 9372 were obtained from Department of Microbiology, Kakatiya University,

Warangal. The bacteria were grown in common selective media nutrient broth (Himedia Pvt, Ltd., Bombay; India) at 37°C and maintained on nutrient agar slants at 4°C.

Chemicals

Podophyllotoxin is purchased from Hi-Theme chemical laboratories, Hyderabad. Other chemicals purchased were of research grade.

Cell Culture

A concentrated stock solution of *Garuga pinnata* extracts was prepared in dimethyl sulphoxide (DMSO) and stored at 20°C until required. Prior to analysis, the samples were diluted in an appropriate growth culture medium with $\leq 0.1\%$ final concentration of DMSO. Human breast cancer cell lines (MCF-7 and MDA-MB-231) were obtained from the American Type Culture Collection (ATCC). MCF-7 cells were cultured in RPMI-1640 medium and MDA-MB-231 cells in Dulbecco's modified Eagle's medium (DMEM) both supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum and maintained at 37°C in a humidified atmosphere of 5% CO₂ in air.

Determination of Anticancer Activity- Preliminary Cytotoxicity Assay on Brine Shrimps (*Artemia Salina*)

Brine shrimp lethality assay of extracts were determined by the method elsewhere.¹² Brine shrimps (*Artemia salina*) were hatched before one day ahead to the experiment in a conical vessel (1L), containing sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were transferred with a sterile glass Pasteur pipette to each vial containing 4.5 ml of brine solution, followed by addition of 0.5 ml of the plant extracts of various concentrations (50, 100, 150 µg/ml) separately and incubated at room temperature for 24 h under the white bright illumination and survival rate of larvae were accounted. Podophyllotoxin (10 µg/ml) and 5% DMSO are used as positive and negative controls, respectively. The reported value is the mean of triplicates.

Determination of LC₅₀ and Mortality

The lethality of the extracts was calculated from the mean survival larvae of extract treated tubes and control using the arithmetic method described elsewhere. Probit analysis was used to determine the Lethal Concentration (LC₅₀) for each extract. The LC₅₀ is defined as the concentration of extract that kills 50% of the shrimps within 24 h.

$$LC_{50} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Determination of Anticancer Activity-Cytotoxicity Assay on Human Cancer Cell Lines

The determination of the *in vitro* cytotoxic potency of the extracts is carried out by the method described by Zhao et al.¹³ In brief, the cells were seeded in a 96-well microtiter plate (Corning, USA) at 2×10^4 cells per well with 100 µL RPMI-1640 growth medium separately, and then incubated for 24 h at 37°C under 5% CO₂ in a humidified atmosphere. Later, the medium was removed while fresh growth medium containing 50, 100 and 150 µg/mL methanolic extract of various parts of *Garuga pinnata* was added. After 3 days of incubation at 37°C under 5% CO₂, the medium was removed and 0.1 mg/mL MTT reagent was added. After incubation for 4 h at 37°C, the MTT reagent was removed before adding 100 µL DMSO to each well and gently shaken. The absorbance was then determined by ELISA reader at 590 nm. Control wells received only the media without the tested samples. The conventional anticancer drug, cisplatin, was used as a positive control in this study. The inhibition of cell growth by various methanolic extracts was calculated as a percent anticancer activity using the following formula: percent anticancer activity $(A_c - A_s / A_c) \times 100\%$, where A_c and A_s referred to the absorbances of control and the sample, respectively.

Anti Bacterial Activity

Preparation of standard bacterial suspensions

The bacterial strains were inoculated into sterilized nutritive broth and incubated at $35 \pm 2^\circ\text{C}$ for 24 h. The turbidity of the resulting suspensions are diluted with same nutritive broth to obtain a transmittance of 25% at 580 nm, this percentage was calculated spectrophotometrically using Bausch & Lomb spectrophotometer comparable to McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml (a stock standard from which a working standard was drawn with concentration of 1×10^8 CFU/ml).

The antibacterial activity of these extracts was carried out according to the method described by Raman with slight modifications.¹⁴ Each selective medium was inoculated with the test organism suspended in nutritive broth. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts of various concentrations and corresponding wells with positive and negative control. The concentration of the methanolic extracts employed at concentrations 25, 50, 75 µg/ml simultaneously, gentamycin sulfate (10 µg/ml) is used as positive control. The test was carried out in triplicate. The plates were incubated at $35 \pm 2^\circ\text{C}$ for 24 h. The inhibition zone diameter was measured in mm.

Statistical Analysis

The data from the experiments of anticancer activities are presented as mean \pm S.E.M (n=3). Student's *t*-test was

used for statistical analysis (SAS software 9.0). Values were considered statistically significant when $P < 0.5$.

RESULTS AND DISCUSSION

As the part of owing to bio integrity and ease in extraction and continuous isolation of natural drugs that are pharmacological active, we have screened *Garuga pinnata* plant parts for the evaluation of anticancer and antibacterial activities. It is important to state that this work represents the first comparative study report on the antibacterial activities of various parts of this plant. Whereas, previous studies of this plant demonstrated cytotoxic activities of Pheophorbide – a & b methyl esters isolated from methanolic crude extract which showed photo-dependent cytotoxic activities against panel of human tumor cell lines.¹⁵ However, the current study describes antioxidant effects of various parts of this plant which would become the first comparative studies. Brine shrimp lethality assay was conducted for the preliminary evaluation of cytotoxic activity of the extracts at various concentrations exploited significant decrease in the survival of brine shrimps relative to the survival of shrimps in the control. Compare to other extracts, leaf and stem bark extracts exhibited highest LC₅₀ values at 50 µg/ml concentration (Table 1). The LC₅₀ Values of these extracts indicates the presence of some potential antitumor and insecticidal compounds.¹⁶ This test was proposed and successfully executed as a standard model to screen the cytotoxic activity of various plants.^{17-19, 12} This assay has great importance for the validation of natural compounds from plant of pharmacological properties, cytotoxic effects, and also used to detect the fungal toxins, heavy metals and pesticides.²⁰ Approximately, 300 novel antitumour and pesticidal natural drugs are isolated in the laboratory at Purdue University, using this bioassay.²¹⁻²³ The anticancer activities of plant extracts are further conformed by performing MTT cell assay tests using various human breast cancer cell lines (MCF-7 and MDA-MB-231) (Table 2 & Figure 1).

Table 1: Cytotoxic effects of *Garuga pinnata* various parts tested

Conc µg/ml	100	150
Leaf	3.3±1.1*	6.0±1.0*
Stem	5.3±0.5	5.3±0.5
Fruit	4.6±0.5	7.3±0.5
Stem bark	5.6±0.5*	8.0±1.0*

Mean ± SEM for n=3 *p<0.5

MTT cell assay revealed that stem bark extract exhibited remarkable cytotoxic activity and showed higher inhibition of growth percentage on MCF cell lines and showed considerable cytotoxicity on MDA-MB-231 cell lines. Whereas, the extracts of fruit, stem and leaves showed moderate cytotoxic activities on the cell lines tested. The methanolic extract of stem bark exhibited concentration dependent growth inhibition of MCF-7 cell

lines and the highest percentage of inhibition was noticed at 150 µg/ml concentration when compared to 50 and 100 µg/ml (Figure 1). However, MDA-MB-231 cell lines showed moderate activity to all plant extracts tested. The percentage of viability of cells is directly proportional to the amount of insoluble purple formazan produced by cleaving tetrazolium ring by succinate dehydrogenase. The cytotoxic activity of stem bark extract indicates presence of phytochemicals that attain different mechanisms which alter metabolic activation of potential carcinogens, hormone production and inhibit aromatase which are required for the cancer cell division.¹³ It has been reported that phenolics are very much potent to reduce the amount of cellular protein and mitotic index, cell proliferation and colony formation of cancer cells.²⁴ Previous studies on pytochemical analysis of *Garuga pinnata* reported that the stem bark methanolic extract possess high amounts the total phenolic and flavanoid content.²⁵ Thus, the present anticancer investigation reports are direct correlation of its activity. Further, the studies also revealed that the stem bark extract possess saponins which is a point to be disused as it exhibits anticancer properties that support the present investigation of cytotoxicity activities.²⁶

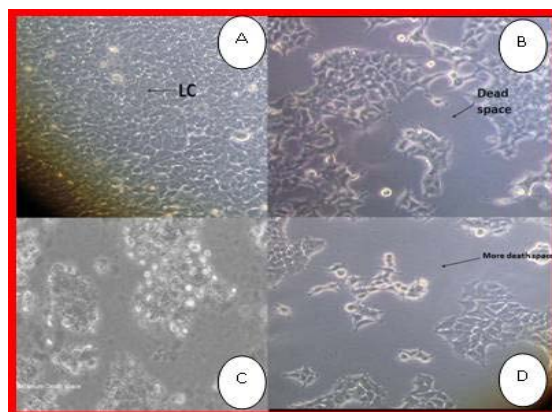


Figure 1: Anticancer activities of *Garuga pinnata* stem bark methanolic extract at 50,100 and 150 µg/ml concentrations tested on human breast cancer MCF cell lines.

- a) Live control of MCF- cell lines, b) Death of MCF-cell lines at 50 µg/ml, c)Death of MCF-cell lines at 100 µg/ml , d) Death of MCF-cell lines at 150 µg/ml

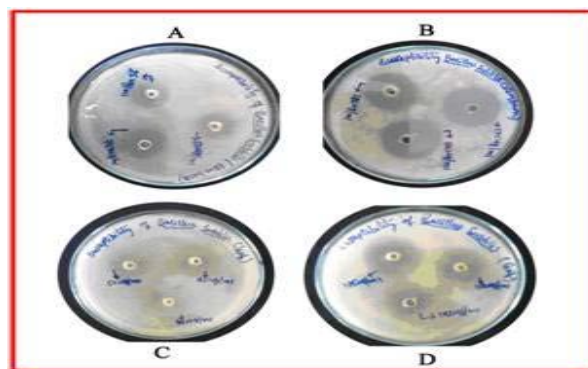


Figure 2: Antibacterial activity of stem bark against *Bacillus subtilis* and *Klebsiella pneumonia*.

Table 2: Anticancer activities of various parts of *Garuga pinnata* on Human breast cancer cell lines at different concentrations tested

MCF-7 Cell line				
Conc µg/ml	Leaf	Fruit	Stem	Stem bark
50	3.7±0.1*	3.5±0.3	2±0.1	5.3±0.4*
100	4.4±0.2*	5±0.2	3.5±0.3	7.4±0.4*
150	6.9±0.2*	6.3±0.3	4.5±0.2	8.3±0.4*
MDA-MB 231 cell line				
50	1.1±0.6	1.5±0.8	3.0±1.7	4.0±2.3*
100	2.0±1.2	1.0±0.5	1.5±0.8	3.5±2.0*
150	2.5±1.4	2.0±1.1	2.5±1.4	3.0±1.7*

Mean ± SEM for n=3 *p<0.5

Table 3: Antibacterial activity of *Garuga pinnata* methanolic extracts at various concentrations compared to known standard

Conc µg/ml	Inhibition zone diameter (mm)												
	Leaf			Stem bark			Fruit			Stem			
	25	50	75	25	50	75	25	50	75	25	50	75	100
S. aureus	3	5	6	4	6	7	3	4	4	3	5	6	18
B. subtilis	6	8	12	8	11	14	4	6	8	3	5	4	15
B. cereus	2	5	6	5	7	8	3	5	6	4	5	7	18
E. coli	5	7	9	7	9	11	5	8	10	3	4	4	29
S. typhi	----	----	----	----	----	----	----	----	----	----	----	----	----
K. pneumonia	6	9	11	9	12	15	5	5	6	4	5	6	21

Antibacterial activity of *Garuga pinnata* leaf, stem bark, fruit and stem methanolic extracts showed a significant zone of inhibitions against various tested organisms. The results were shown as the inhibition of zone diameter. Among, plant extracts tested, stem bark extract reported highest antibacterial activity against various gram positive and gram negative bacterial strains. *Bacillus subtilis* and *Klebsiella pneumonia* exhibited highest susceptibility and the zone of inhibition diameter was noticed are 14 and 15 respectively (Table 3 & Figure 2). However, leaf extract are also produced comparable inhibition zone diameter. Whereas, the fruit and stem extracts produced very less inhibition zone diameters. Garuganin-I and II a known diary heptanoids isolated from stem bark of this plant exhibits structural similarity with rifamycin SV, a typical ansamycin antibiotic suggest an analogous mechanism for antibacterial action.²⁷⁻²⁸ The study reveals that *S. typhi* was found to be resistant towards leaf, fruit and stem extracts but showed considerable susceptibility towards stem bark extract. The speculated reasons for the highest antibacterial activity of stem bark extract might be due to presence of alkaloids and flavanoids which are generally associated with the activity.^{29-30, 25}

CONCLUSION

The methanolic extract of *Garuga pinnata* stem bark have been noticed significant anticancer activity on MCF-7 human breast cancer cell lines and also exhibited antibacterial properties at various concentrations tested. Therefore, this investigation is a direct correlation on usage of stem bark extract as folklore for treatment of different ailments.

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