Int J Pharma Res Health Sci. 2018; 6 (3): 2610-14
DOI:10.21276/ijprhs.2018. 2018. 03.08
K Padmalochana
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Antibacterial, Anticancer and Antioxidant Activity of *Cassia auriculata* Leaves Methanolic, Petrolium Ether and Ethyl Acetate Extracts

K Padmalochana *
Head of the Department of Biochemistry, Sri Akilandeswari Women's College, Wandiwash – 604408, Tamil Nadu, India

ARTICLE INFO

Received: 24 Apr 2018
Accepted: 12 May 2018

ABSTRACT

The Anticancer, antioxidant efficacy and antibacterial activity test of leaves of *Cassia auriculata*, were performed. Antioxidant activity of all three extracts of leaves of *Cassia auriculata* was investigated using DPPH radical scavenging method. Antioxidant efficacy of methanolic and ethyl acetate extracts were found to be higher and comparative with standard ascorbic acid at concentration of 2- 10µg/ml. Antibacterial activity test showed some antibacterial potency of the extracts even though it was very lower as compared to the standard antibiotics. Methanolic extract showed the highest degree of antbacterial activity against *E. coli, Salmonella sp* and *staphylococcus aureus* as compared to the other extracts. From this study we can concluded that this plant consist several phytochemicals. Also this plant has both antibacterial and antioxidant property though they were comparatively lowers than that of the standard used in the study.

Key words: *Cassia auriculata*, Antibacterial, anticancer, antioxidant activity

1. INTRODUCTION

The scientific classification of Cassia auriculata is

Kingdom	:	Plantae
Order	:	Fabales
Family	:	Leguminosae
Subfamily	:	Caesalpinioideae
Genus	:	Cassia
Species		auriculata

Cassia auriculata is the very good medicinal plant having many pharmacological applications such as antipyretic activity [1], antilipidemic, antispasmodic, antidiabetic and antiviral activities [2-4], hypolipidemic and antihyperglycemic

Corresponding author *
Dr K Padmalochana
Head of the Department of Biochemistry, Sri Akilandeswari Women's College, Wandiwash – 604408, Tamil Nadu, India
Email.id: kpadmalochana@gmail.com

activity [5, 6], hepatoprotective activity in *in-vivo* against ethanol induced hepatotoxicity [7], nephroprotective activity in cisplatin and gentamicin-induced renal injury in animals [8], whole plant extract used for antibacterial activity against *Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli,* [9] aqueous extract of *Cassia auriculata* leaves used for anthelmintic activity against earthworms (*Eisenia fetida*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*) [10]. Anticancer activity performed against human larynx carcinoma (Hep-2) cell lines and in human breast adenocarcinoma (MCF-7) [11], methanolic extract in rats was evaluated for its immunomodulatory activity [12]. In this present investigation we have analysed the antibacterial activity of *Cassia auriculata* methanolic, petroleum ether and ethyl acetate extract against *Bacillus subtilis, Klebsiella pneumoniae* and *Escherichia coli*. We determined the antioxidant activity of plant extract using free radical scavenging assay of DPPH assay and hydroxyl activity. And finally we have analysed the anticancer activity of *Cassia auriculata* methanolic, petroleum ether and ethyl acetate extract on liver cancer cell lines.

2. MATERIALS AND METHODS

Collection and preparation of *Cassia auriculata* extract

The leaves of *Cassia auriculata* (Figure 1) were collected from local area of Wandiwash, Thiuvanamalai district. The identification of *Cassia auriculata* was confirmed and authenticated. The leaves are dried and ground into powder using porcelain mortar and pestle. The prepared plant powder has been extracted using soxhlet apparatus. All the extracts obtained were then concentrated in rotary flash evaporator and dried in a vacuum oven at 50 °C, and yield value for each extract was calculated.

Fig 1: *Cassia auriculata*

Biomedical applications of plant extract

DPPH radical scavenging activity test

Antioxidant activity was measured by DPPH radical scavenging method (Ayoola et al., 2008). Solutions of concentrations 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 µg/ml were prepared for each extract. Freshly prepared 10 ml DPPH solution (1 mM) was mixed with 20 ml of different samples (2 – 10) µg/ml. Ascorbic acid solutions of same concentrations 2µg/ml ─ 10 mg/ml in methanol were prepared and used as positive control for the radical scavenging activity test. Fifteen minute later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH free radical was calculated using the following equation:

$$\%\ \text{of radical scavenging activity} = \frac{(\textbf{Abs}\ \text{control} - \textbf{Abs}\ \text{sample})}{(\textbf{Abs}\ \text{control})} \times 100$$

Where,

Abs control = Absorbance of DPPH solution

Abs sample = Absorbance of extracts and ascorbic acid solutions

Antibacterial activity test

Agar well diffusion method

Qualitative assay of antibacterial activity of plant extracts was performed by standard methodology i.e. agar well diffusion method. The antibacterial medium Muller Hinton Agar was used in this study. Different volumes of crude plant extracts were dissolved in distilled water (25-150 µg/ml). Pathogenic bacteria were grown in Nutrient broth for 24 hr and swapped on the petriplates containing Muller Hinton Agar. In MHA agar plate, about 6 mm diameter wells were made by gel puncture. Diluted extracts with different concentration (25 -150 µg) were applied into the well and the plates were incubated at 37°C for 24 hr. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Anticancer activity of *C. auriculata*

Cells viability test was done by the MT(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazoliumbromide) assay is s colorimetric analysis based on the measuring the activity of cellular enzymes that living cells were reduce the yellow MTT dye into insoluble purple color formazan. Cells were plated and grown with different concentration of plant extracts and incubated for 24 hours in CO2 atmosphere. After 24 hours treatment, MTT was added in each well and incubated at 37C for 4 hours in 5% CO2 chamber. Then the medium was removed and washed with Phosphate buffer solution. Then, DMSO was added to each well which dissolve the insoluble formazan crystals into colored solution. The intensity of the colored solution was measured using ELISA microplate reader at 570 nm. The results were expressed as the percent optical density of treated cells to that of the control cells. The 50 % of inhibitory concentration value (IC_{50}) of the extracts was identified for normal untreated cell line. Commercial anticancer drug Doxorubicin was used as a control. The assay was performed in triplicate for each extracts.

$$\%\ \text{Cell viability} = 1 - \frac{\text{Absorbance for treated cells}}{\text{Absorbance for control cells}} \times 100$$

3. RESULTS AND DISCUSSION

Antioxidant (free radical scavenging) activity

In the present study the antioxidant activity test was found to be positive for each extract which can be attributed to the

presence of phenols and flavonoids as shown by the phytochemical screening test(Figure 2-7)[13]. The comparative study showed the higher antioxidant activity of methanol and ethyl acetate extract than that of petroleum ether extract. The methanol and ethyl acetate extract showed the presence of both flavonoids and phenols while petroleum ether extract contained only phenol. The presence of both types of antioxidant, flavonoids and phenolic compounds in ethyl acetate and methanol extracts can be concluded for higher antioxidant activity of these extracts as compared to petroleum ether extract.

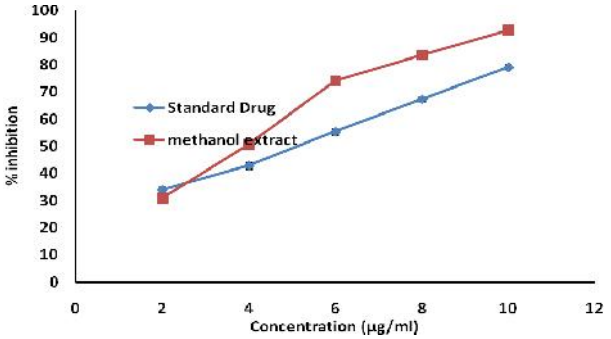

Fig 2: DPPH free radical scavenging activity of methanol extracts and standard at different concentrations

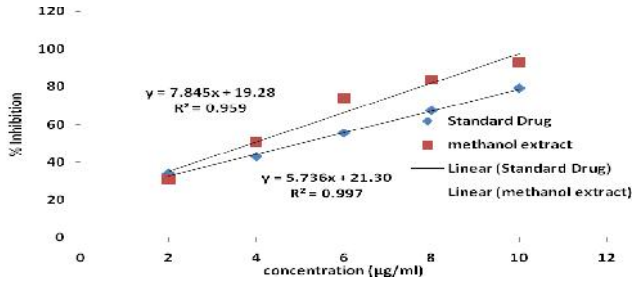

Fig 3: Regression coefficient measurement to measure the linearity of the curve

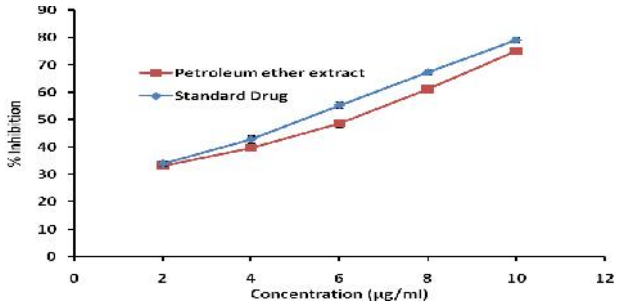

Fig 4: DPPH free radical scavenging activity of petroleum ether extracts and standard at different concentrations

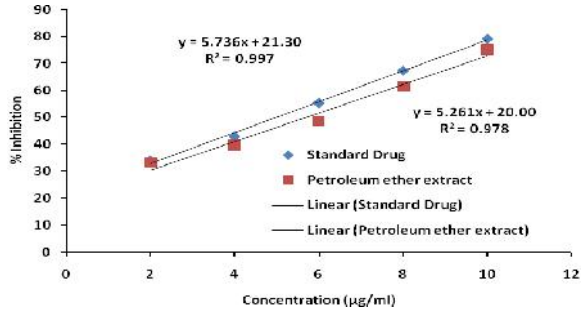

Fig 5: Regression coefficient measurement to measure the linearity of the curve

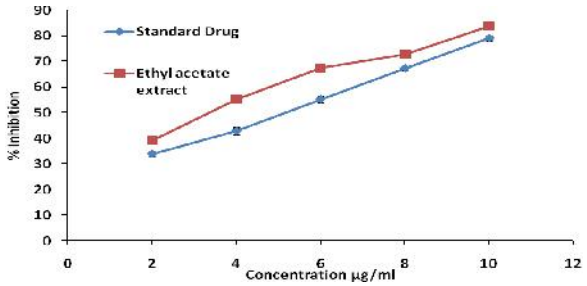

Fig 6: DPPH free radical scavenging activity of ethyl acetate extracts and standard at different concentrations

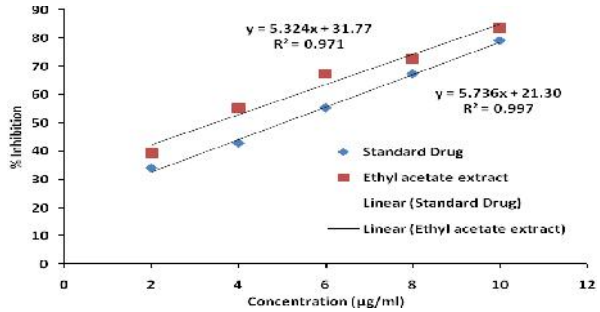

Fig 7: Regression coefficient measurement to measure the linearity of the curve

Antibacterial activity of *C. auriculata*

The antimicrobial activity of three extracts of Cassia auriculata was tested under in vitro conditions by agar well diffusion. Antibacterial activity of methanol, ethyl acetate and petroleum ether extract were evaluated against three bacterial species *Klebsiella pneumoniae*, *Bacillus subtilis*, and *E.coli*, which are known to cause diarrhea dysentery, abscesses, wounds and other infections in human. To evaluate the antibacterial efficacy of different extracts against standard antibiotics; zone of inhibition (Table 2-4) were measured. Methanolic extract showed greater zone of inhibition for *E. coli*, *Pseudomonas aeroginosa* than that of ethyl acetate extract, whereas petroleum ether extract showed the least antibacterial effect. In case of *E. coli* ethyl acetate extract showed greater zone of inhibition than that of petroleum ether extract whereas methanolic extract showed least antibacterial activity (Figure 8-10).

Table 2: Antibacterial activity of methanolic extract of *C. auriculata*

Conc (µg/ml)	Zone of inhibition (mm in diameter)		
	E.coli	***K. pneumoniae***	***B. subtilis***
25	16±0.36	15±1.09	13±0.12
50	18±0.18	18±0.29	15±2.09
100	25±0.15	23±2.12	22±1.15
150	30±0.72	26±0.67	25±0.19
AMP	23±1.19	22±0.22	19±1.18

AMP – Amphicillin

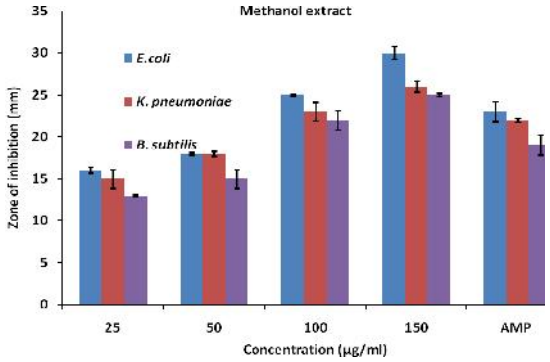

Fig 8: Antibacterial activity of methanolic extract of *Cassia auriculata*

Table 3: Antibacterial activity of ethyl acetate extract of *Cassia auriculata*

Conc (µg/ml)	Zone of inhibition (mm in diameter)		
	E.coli	***K. pneumoniae***	***B. subtilis***
25	17±1.36	14±1.09	14±1.02
50	20±2.25	15±1.19	17±2.09
100	25±0.75	18±2.12	19±1.75
150	29±0.72	20±1.97	22±1.69
AMP (100 µg)	23±1.19	22±1.32	19±1.98

AMP – Amphicillin

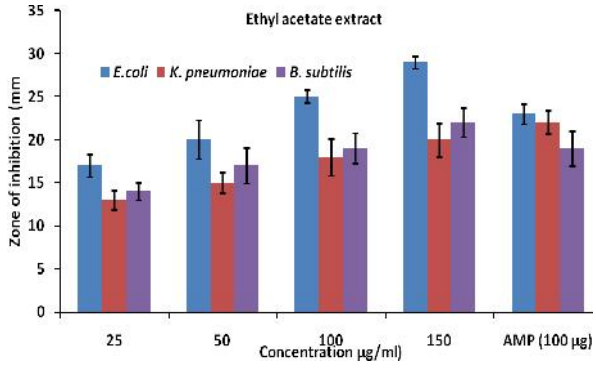

Fig 9: Antibacterial activity of ethyl acetate extract of *Cassia auriculata*

Table 4: Antibacterial activity of petroleum ether extract of *Cassia auriculata*

Conc (µg/ml)	Zone of inhibition (mm in diameter)		
	E. coli	***K. pneumoniae***	***B. subtilis***
25	11±0.36	12±1.09	07±0.12
50	13±0.18	16±0.29	09±2.33
100	15±0.15	20±2.12	12±1.45
150	18±0.72	23±0.67	15±2.19
AMP (100 µg)	23±1.19	22±0.22	19±1.18

AMP – Amphicillin

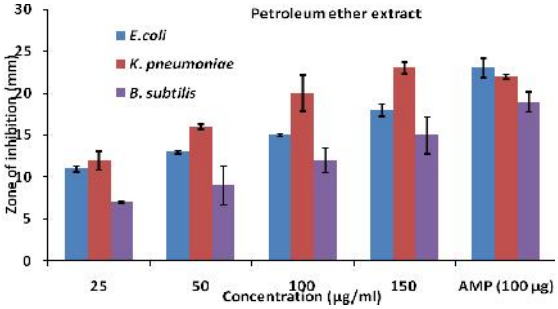

Fig 10: Antibacterial activity of petroleum ether extract of *Cassia auriculata*

Anticancer activity of *Cassia auriculata*

The medicinal herb *C. auriculata* leaves showed anticancer activity against HepG2 (Liver cancercell line) cell lines (Table 1).In the present study, the treatment with methanol, ethyl acetate and petroleum ether extracts suppressed the cell viability up to 50% at 200µg/ml against both cell lines (Figure1-3).Plant extracts shows more significant activity as compared to the positive control. The extract showed significant inhibition in the cell viability in a dose dependent manner. The treatment with ethanol extract against HepG2 (Liver cancer cell line) cell lines significantly decrease the viability of cells at 200g/ml when compared other extracts. The cells were contact with 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, and 300µg/ml of extract showed decreased number of cell viability. The results indicate that extracts of *C. auriculata* has an anticancer activity in HepG2 cell lines. The maximum cytotoxic effect was observed in methanol extract

Table 5: Anticancer activity of extracts *C. auriculata* leaves against HepG2 (Liver cancer cell line)

concentration (µg/ml)	Cell Viability %			
	Standard Drug	**Petroleum ether extract**	**Methanol extract**	**Ethyl acetate extract**
50	92.53±0.99*	98.12±0.9*	94.21±1.14**	96.55±1.25**
100	76.44±1.27**	92.56±1.09*	84.87±1.65**	91.82±1.64*
150	51.33±1.14*	79.78±1.25**	66.65±1.25**	65.65±1.45**
200	29.65±0.64*	57.19±1.16**	52.15±0.85***	54.25±0.82**
250	19.23±0.81**	40.54±1.15**	42.15±1.09**	45.77±1.9**
300	10.89±0.47*	26.38±0.95**	21.64±0.95***	23.61±0.95**

* **p< 0.05,**** **p < 0.01,*******p < 0.001** value are considered statistically significant (BMRT)

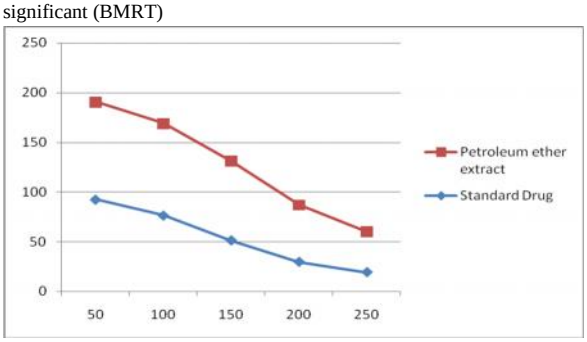

Fig 11: Anticancer activity of petroleum ether extract of *Cassia auriculata*

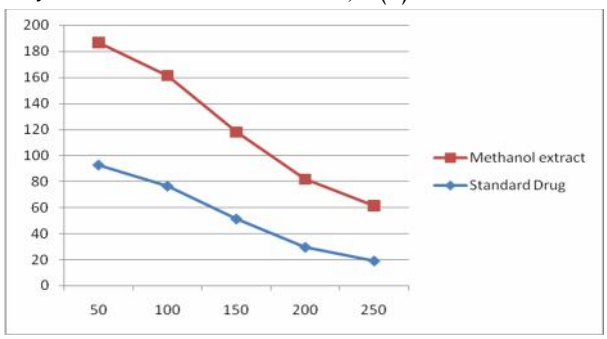

Fig 12: Anticancer activity of methanol extract of *Cassia auriculata*

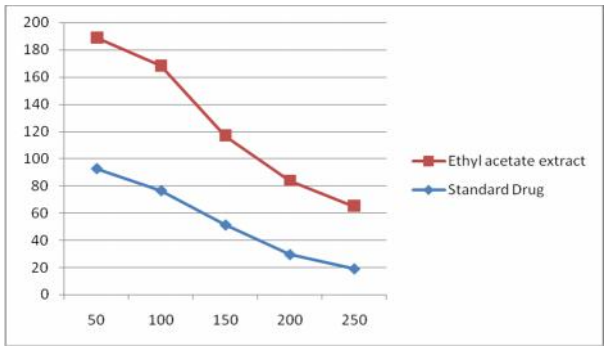

Fig 13: Anticancer activity of Ethyl acetate extract of *Cassia auriculata*

4. CONCLUSION

Current days, there is an urgent need for improved management and investigation of such plants. Moreover those natural antioxidants can have potential advantages among various diseases with oxidative stress and for that leaves of *Cassia auriculata* may be one of the alternatives. With more resources and time, further investigation of chemical constituents of leaves of *Cassia auriculata* and other poorly studied plants can be revealed. Therefore more of the researches and studies are required as there is a large untapped reservoir waiting to be investigated.

5. REFERENCES

1. Vedavathy S and Rao K N. Antipyretic activity of six indigenous medicinal plants of Tirumala hills. J. Ethanopharmacol 1991; 33: 193-196.
2. Sivaraj A, K Devi, S Palani, Kumar P V, Kumar B.S. and David E. Antihyperglycemic and antihyperlipidemic effect of combined plant extract of *Cassia auriculata* and Aegle marmelos in streptozotocin-induced diabetic albino rats. Int. J. Pharm. Tech. Res 2009; 1: 1010-1016.
3. Dhar M L, Dhar M M, Dhawan B N, Mehrotra B N and Ray C. Screening of Indian plants for biological activity: I. Indian J. Exp. Biol 1968; 16: 232-247.
4. Pari L and Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in Streptozotocin diabetic rats. Singapore Med. J. 2002; 43: 617-621.
5. Gupta S, Sharma S B, Prabhu K M and Bansal S K, Protective role of *Cassia auriculata* leaf extract on hyperglycemia-induced oxidative stress and its safety evaluation. Indian J. Biochem. Biophys. 2009; 46: 371-377.
6. Kumar R S, Ponmozh M, Viswanathan P and Nalini N. Effect of *Cassia auriculata* leaf extract on lipids in rats with alcoholic liver injury. Asia Pac. J. Clin. Nutr. 2002; 11: 157-163.
7. Rajagopal S K, Manickam P, Periyasamy V and Namasivayam N. Activity of *Cassia auriculata* leaf extract in rats with alcoholic liver injury. J. Nutr. Biochem. 2003; 14: 452-458.
8. Annie S, Rajagopal P L and Malini S. Effect of *Cassia auriculata* Linn. root extract on cisplatin and gentamicin-induced renal injury. Phytomedicine 2005; 12: 555-560.
9. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006 ;6(1):35.
10. Kosalge S B and Fursule R A. Investigation of anthelmintic potential of some plants claimed by tribals of satpuda hills. Int. J. PharmTech Res. 2009; 1: 68-72.
11. Prasanna R, Harish C C, Pichai R, Sakthisekaran D and Gunasekaran P. Anti-cancer effect of *Cassia auriculata* leaf extract In vitro through cell cycle arrest and induction of apoptosis in human breast and larynx cancer cell lines. Cell Biol. Int. 2009; 33: 127-134.
12. Chakraborthy G S. Evaluation of immunomodulatory activity of *Cassia auriculata* Linn. J. Herbal Med. Toxicol 2009; 3: 111-113.
13. Anushia C, Sampathkumar P, Ramkumar L. Antibacterial and antioxidant activities in Cassia auriculata. Glob J Pharmacol. 2009;3(3):127-30.

Conflict of Interest: None

Source of Funding: Nil