IN VITRO ANTICANCER ACTIVITY AND XANTHINE OXIDASE INHIBITORY PROPERTIES OF *Justicia beddomei*

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ABSTRACT: The plant *Justicia beddomei* has numerous therapeutic utility in folk medicine. In the present study the *in vitro* anticancer and xanthine oxidase inhibitory (XOI) activity was investigated. The dried aerial parts of *J. beddomei* were successively extracted with methanol. The extract was subjected to MTT colorimetric assay in HeLa and MCF-7 cell lines and its xanthine oxidase inhibitory activity determined by using standard allopurinol. The XOI activity of the methanolic extract increased in a dose dependent manner and the MTT assay showed moderate cytotoxicity against the cell lines. The results obtained in the present study indicate that the methanolic extract of *J. beddomei* contains phytochemicals which has anticancer and XOI activities.

KEYWORDS:- HeLa, MCF-7, *Justicia beddomei*, Anticancer, Xanthine oxidase

1. INTRODUCTION

The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical (1). A variety of bioactive compounds and their derivatives has been shown to inhibit carcinogenesis in a number of experimental systems involving initiation, promotion and progression (2, 3). Plants contain abundant quantities of these substances and have consistently been shown to be associated with a lower risk of cancers at almost every site (4). Efforts, therefore, are being made to identify naturally occurring anti-carcinogens which would prevent, slow or reverse the cancer induction and its subsequent development (5). It has been estimated that only 5-15% of 250,000 species of higher plants have been screened systematically for natural bioactive compounds. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources. To study new therapeutic approaches, cell lines are used to investigate novel compounds and their effects on the tumor cells (6, 7).

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Xanthine oxidase (XO) is a member of a group of enzymes known as molybdenum iron- sulphur flavin hydroxylases and it catalyses the oxidative hydroxylation of purines and subsequent reduction of oxygen at the flavincentre with generation of reactive oxygen species (ROS), either superoxide anion radical or hydrogen peroxide (the oxidative half-reaction)(8). It converts hypoxanthine to xanthine and xanthine to uric acid in our body. Any deficiency or excess of a specific metabolite in the body may cause diseases, or at least symptoms of the diseases (9).

Xanthine oxidase inhibitors (XOI) are agents that directly inhibit the synthesis of uric acid *in vivo* and thus these drugs should be given prime importance in the therapeutic approach for the treatment of gout and hyperuricemia (10). However, allopurinol a widely used XO inhibitory agent suffers from many side effects such as hypersensitivity reactions and renal toxicity (11). Certain active constituents present in crude plant extracts like flavonoids and polyphenolic compounds have been reported to possess XOI as well as free radical scavenging and inhibition of oxidative enzymes (12, 13). Thus, these findings open up the possibility of developing newer natural compounds from medicinal plants that are free of any unwanted side effects.

In Cancer therapy, which involves greater protein catabolism leading to an increase in production of an end product uric acid which on accumulation can lead to conditions like gouty arthritis which involves deposition of urate crystals in the synovial joint causing inflammation. Hyperuricemia may be an early manifestation of the carcinogenic process (14). Anti-cancer drugs which are having the ability to inhibit xanthine oxidase can prove to be beneficial in cancer therapy as ultimately the drugs can inhibit xanthine oxidase and their by decrease the deposition of uric acid and prevent gouty arthritis.

The genus *Justicia* comprises about 300 species world over and nearly 50 species occur in lndia (15). About 20 species have been chemically investigated and reported to contain lignans, triterpenoidal glycosides and amide (16). The plants of *Justicia* genus are rich sources of bioactive lignans particularly arylnaphthalides (17, 18). The plant has been found to be having anti-diabetic, anti-inflammatory and anthelmintic properties (19, 20). The plant is distributed in the hilly regions of Kerala. Various parts are widely used in folk medicine as an expectorant, diuretic, antispasmodic, depurative and styptic. Leaves are used in diarrhoreal bleeding and irritative cough. Flowers are used in ophthalmic preparations and the roots along with the leaf juice are used in pthisis, cough, and asthma (21). Along with turmeric it is used in an anti-ageing cream to prevent wrinkles in the skin. However no such reports on XOI and anti-cancer studies are reported till date.

Hence the methanolic extract of the aerial part of *Justicia beddomei* which exhibited significant antioxidant activity (22) was evaluated for its *in vitro* XOI and anticancer activities.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material was collected from hilly regions of Kerala, India. The plant was botanically identified by Dr. V. Chelladurai, Research Officer Botany, (Rtd) CCRAS, Government of India. A voucher Specimen has been kept in the department of chemistry (NCP/CH/ PS/JB01), National College of Pharmacy, Calicut.

2.2. Chemicals and Cell lines

All the chemicals of superior grade were purchased from SD Fine Chemicals and cell lines in National Institute of technology was used for anticancer study.

2.3. Extraction

The aerial parts of *J. beddomei* were dried, grounded into fine powder and sieved through No. 20 mesh sieve. About 100 g of powdered aerial part was defatted and subjected to soxhlet extraction with 500 ml of methanol. The percentage yield of methanol was 8.8% w/w.

2.4. MTT assay

Sensitivity of MCF-7 and HeLa cells to methanolic extract of *J. beddomei* was determined individually by the MTT colorimetric assay. Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 h at 37°C and in 5% CO_2. Both cell lines were exposed to the extract. Medium was changed at 48 hrs intervals. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of test extract in microtitre plates and kept for incubation at 37°C in 5% CO_2 incubator for 72 hour and cells were periodically checked for granularity. The solvent DMSO treated cells served as control. Cells were then treated with MTT reagent (20 µl/well) for 4 h at 37°C and then DMSO (200 µl) was added to each well to dissolve the formazan crystals. The optical density (OD) was recorded at 492 nm in a microplate reader.

Percentage of residual cell viability was determined as

[1− (OD of treated cells/OD of control cells)]×100.

2.5. *In vitro* xanthine oxidase inhibitory activity

The xanthine oxidase inhibitory activity was assayed spectrophotometrically under aerobic conditions

using xanthine as the substrate (26). The extract and the standard drug allopurinol (1 mg/ml) were prepared by dissolving in DMSO initially and then made up to the required volume with potassium dihydrogen phosphate buffer, pH 7.5. The assay mixture (250 µl) of extracts at different concentrations (2.5-40 µg/ml) consisted of 175 µl of potassium dihydrogen phosphate buffer and 75 µl of xanthine oxidase enzyme (1.33U/ml) solution. After pre-incubation at 37°C for 10 min 500 µl of 0.15mM substrate (xanthine) was added, mixed and incubated at 37°C for 30 min. Then 200 µl of 1N HCl was added to arrest the reaction and the absorbance was measured at 290 nm against blank (buffer solution) and a control reaction was carried out without the test sample (27). The percentage inhibition was calculated using the following formula,

$$I\% = \frac{Ac - At}{Ac} x100$$

Where A_c is the absorbance of control reaction and A_t is the absorbance of test reaction. The assay was done in triplicate for each concentration. Allopurinol (2.5-40 µg/ml) was used as standard.

3. RESULTS AND DISCUSSION

3. 1. MTT assay

In the present study, the cytotoxic effect of methanolic plant extract on MCF-7 and HeLa cells was evaluated by MTT assay. MTT assay is a well-established *in vitro* method for cytotoxicity against cancer cell lines and non-cancer cell lines. This assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethyl thiazol-2-yl)-2, 5 diphenyl tetrazolium bromide (MTT), which is measured spectrophotometrically (28). This formazan production is inversely proportional to the degree of cytotoxicity (29). Different dilutions of extracts were treated and IC_{50} values were calculated. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work. In the present study methanolic extract on both the cell lines showed moderate activity. The results are tabulated in Table 1.

Table 1. MTT assay

Conc (µg/ml)	% Cell inhibition			
	MCF-7		HeLa	
	MEJ	Tamoxifen	MEJ	Tamoxifen
12.5	15.06±0.7	10.17±0.5	17.50±0.4	11.08±0.3
25	26.10±0.8	32.21±0.4	38.44±0.1	28.42±0.3
50	39.35±0.2	65.50±0.0	44.56±0.3	59.54±0.1
100	46.50±0.7	80.52±0.5	57.65±0.4	68.46±0.7
200	53.21±0.5	85.24±0.2	60.77±0.2	82.56±0.2
IC_{50}	152.16*	38.42	70.77*	42.36

MEJ- *J. beddomei* methanolic extract; All values determined were mean±SEM; *n*= 3; *P < 0.05 when compared with standard.

Table 1. *In vitro* xanthine oxidase inhibitory activity

Conc.(µg/ml)	Percentage Inhibition	
	MEF	Allopurinol
2.5	29.18±0.24	32.50±0.22
5	39.80±0.18	57.72±0.14
10	59.91±0.17	68.85±0.12
20	68.50±0.21	85.10±0.15
40	81.22±0.18	95.25±0.28
IC_{50}	8.37±0.21*	4.23±0.17

MEJ- *J. beddomei* methanolic extract; All values determined were mean±SEM; *n*= 3; *P <0.05 when compared with standard.

3.2. *In vitro* xanthine oxidase inhibitory activity

The methanolic extract was assayed for their XOI activity at different concentrations in the assay mixture. From the results it is evident that the methanolic extract (IC_{50} = 8.37±0.21) exhibits significant inhibitory activity. These results (Table 2) when compared with the standard (IC_{50} = 4.18 ± 0.17) allopurinol showed significant ($p<0.05$) inhibitory activity. Flavonoids are a group of poly phenolic compound which has been proved to possess xanthine oxidase inhibitory activity (30). Thus the presence of flavonoids and phenolic in the crude extract would have contributed towards the inhibitory activity.

4. CONCLUSION

The current study has demonstrated that the methanolic extract of aerial parts of *Justicia beddomei* Linn. contained compounds capable of inhibiting XO and inducing cytotoxic effects on cell lines. Furthermore, these compounds might be helpful in treating various cancer conditions and resulting gouty arthritis. However, further investigations such as isolation of active compounds present in the extract and *in vivo* studies are necessary to identify the specific chemical entity for clinical use.

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REFERENCE

1. Sundaram S, Verma SK and Dwivedi P. *In vitro* cytotoxic activity of indian medicinal plants used traditionally to treat cancer. *Asi. J. Pharmace. Clin. Res* 2011; 4(1): 27-29.
2. Ho CT, Osawa T, Huang MT and Rosen RT. (Eds.). (1994) Food Phytochemicals for Cancer Prevention II Teas, Spices, and Herbs. *ACS Symposium 547*, Washington DC. American Cancer Society.
3. Huang, M.T., Osawa, T., Ho, C.T and Rosen, R.T. (Eds.). (1994) Food Phytochemicals for Cancer Prevention I Fruits and Vegetables, *ACS Symposium 546*, Washington DC. American Cancer Society.
4. Steinmetz K and Potter JD. A review of vegetables, fruit and cancer. Epidemiology Cancer Causes Control 1991; 2: 325–357.
5. Chuang SE, Kuo ML, Hsu CH, Chen CR, Lin JK, Lai GM, et al. Curcumin-containing diet inhibits diethyl nitrosamine-induced murine hepato carcinogenesis. *Carcinogenesis* 2000; 21: 331–335.
6. Cragg GM and Newman DJ. Discovery and development of antineoplastic agents from natural sources. *Cancer invest* 1999; 17: 153-163.
7. Cragg GM, Newman DJ and Snader KM. Natural products in drug discovery and development. *J. Nat. Prod.* 1997; 60: 52-60.
8. Lacy F, Gough DA and Schmid-schonbein GW. Role of xanthine oxidase in hydrogen peroxide production. *Free Rad. Bio. Med* 1998;25(6):720–727.
9. Borges F, Fernandes E and Roleira F. Progress towards the discovery of xanthine oxidase inhibitors. *Cur. Med. Chem*. 2002; 9(2):195–217
10. Unno T, Sugimoto A and Kakuda T. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia* Speciosa (L.) Pers. *J. Ethnopharmacology* 2004; 93:391–395.
11. Umamaheswari M, Asokkumar K, Sivashanmugam AT, Subhadradevi V and Ravi TK. *In vitro* xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* Roxb. *J. Ethnopharmacology* 2009; 124:646–648.
12. Chang CC, Yang MH, Wen HM and Chern JC. Estimation of total flavonoids content in *propolis* by two complementary calorimetric methods. *J Food Drug Anal.* 2002; 10(3):178–182.
13. Costantino L, Albasini A, Rastelli G and Benvenuti S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med* 1992; 58:342–344.
14. Boffetta P, Nordenvall C, Nyrén O and Ye W. A prospective study of gout and cancer. *Eur, J. Cancer Prev.* 2009; 18(2):127- 32.
15. Rajasekhar D, Vanisree M and Subbaraju GV. *Justicia* Lignans: Two new aryl naphthalide lignans from *Justicia neesii* Ramamoorthy. *Ind. J. Chem* 1999; 38:713–17.
16. Subbaraju GV, Rajasekhar D, Kavitha J, Hsu FL and Chang K. *Justicia* lignans: Part 10-Synthesis

of tiruneesiin,the first neolignan from *Justicia* species. *Ind. J. Chem*. 2007; 46B:357–9.

17. Subburaju GV, Kumar KKK, Raju BL and Pillai, KR. Justiciresinol, A New Furanoid lignan from *Justicia glauca*. *J. Nat. product* 1991; 54:1639–41.

18. Srinivasa U, Rao VJ, Krupanidhi AM and Divakar K. Antidiabetic activity of *Justicia beddomei* leaves in alloxan induced diabetic rats. *J. Res. Educ. Ind. Med.* 2008; 45–8.

19. Srinivasa U, Krupanidhi AM, Rao VJ and Shanmukhappa. Anthelmintic activity of leaves of *Justicia beddomei*. *Ancient Science of Life* 2007; 26(3): 1–3.

20. Warrier PK, Nambiar VPK and Ramankutty C. Indian medicinal Plants: A Compendium of 500 Species. New Delhi: Orient Longman. 1996; pp.268.

21. Akash Marathakam,Kannappan N et al . Studies on phytochemical and *In vitro* antioxidant potential of *Justicia beddomei* (Clarke) Bennett .*Free Rad. Antioxidant* 2012; 2(4) 26-31.

22. Sankara AJ, Naresh KL and Animisha M. *In vitro* anti-cancer activities of few plant extracts against MCF-7 and HT-29 cell lines VSPK. *Int. J. Pharma Sci.* 2013; 3(2): 185-188.

23. Jack DB. The MTT assay to evaluate chemosensitivity. Chemosensitvty Vol 1, Humana Press Inc.Totowa , NJ 2005; 5:69-77.

24. Taylor RSL, Manendhar NP, Hudson JB and Towers GHN. Antivral activities of Nepalese medicinal plants. *J Ethnopharmacology* 1996; 52:157-63.

25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. immun. Methods* 1983; 65:55-63.

26. Berridge MV, Herst PN and Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. In: El-Gewely MR. Biotechnology Annual review. Amsterdam, Elsevier: 2005; 127-152.

27. Nguyen MTT, Awale S, Tezuka Y, Tran LQ, Watanabe H and Kadota S. Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. *Biol. Pharm. Bull* 2004; 27:1414–1421.

28. de Puerta, L.A., Forder, R.A and Hoult, J.R.S. Inhibition of leukocyte eicosanoid generation and radical scavenging activity by Gnaphalin, a lipophilic flavonol isolated from *Helichrysum picardii*. *Planta Med*. 1999; 65:507–11.

29. Cardellina, J. H., Fuller, R.W., Gamble, W.R., Westergard, C and Boswell, J. (1999) Evolving strategies for the selection dereplication and prioritization of antitumour and HIV-inhibitory natural products extracts. In: Bohlin L, Bruhn JG. (eds.) Bioassay methods in natural product research and development. Dordrecht: Kluwer Academic Publisher; p. 25-36.

30. Lio M, Moriyama A, Matsumoto Y, Takaki N and Fukumoto M. Inhibition of xanthine oxidase by flavonoids. *J. Agri. Bio. Chem*. 1985; 49:2173–2176.