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In vitro antioxidant and cytotoxic activity of Zanthonitrile isolated from *Zanthoxylum alatum*

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ABSTRACT

The present study evaluates the antioxidant and cytotoxic activity of Zanthonitrile isolated from *Zanthoxylum alatum* leaves. The structure of Zanthonitrile [{4-[(3-Methyl-2-buten-1-yl) oxy] phenyl} acetonitrile] was elucidated form the data obtained from UV, IR, Mass, and NMR. The *in vitro* antioxidant activity of Zanthonitrile was estimated by standard 1, 1-diphenyl-2-picrylhydrazil radical (DPPH) scavenging assay method. *In vitro* cytotoxicity was determined in Ehrlich Ascites Carcinoma (EAC) cells by MTT assay. The compound exhibited antioxidant activity in a dose dependent manner. The IC_{50} value of Zanthonitrile for DPPH was estimated to be 7.86 ± 0.23 µg/mL. Zanthonitrile showed satisfactory cytotoxic potential in MTT assay with the IC_{50} value 57.28 ± 0.64 µg/mL. Satisfactory results of both the studies correlate each other and further investigations will focus on *in vivo* models and cell cycle to determine the role on intrinsic and extrinsic apoptotic pathways.

INTRODUCTION

Zanthoxylum alatum Roxb. (Family: Rutaceae) is an evergreen plant of the Himalayan regions in India commonly known as Tejphal (Hindi) and Timur (Nepal) (Tiwary *et al.*, 2007; Singh and Singh, 2011).

Nepalese traditionally used the fruit decoction in abdominal pain, bark extract as cholera, diabetes and asthma. Pickles from the fruits are used by Nepalese for treating cold & cough, tonsillitis, headache, fever and high altitude sickness (Geweli and Awale, 2008). Different parts of the plant are traditionally used as a stomachic, carminative, disinfectant, antiseptic. The plant is also used for the treatment of fever, dyspepsia, cholera, anthelmintic, general debility (Jain *et al.*, 2001; Kalia *et al.*, 1999). It has been proven that the plant have the antibacterial, antifungal, anthelmintic and larvicidal activities (Tiwary *et al.*, 2007). As well as hepatoprotective (Ranawat *et al.*, 2010), antinociceptive, anti-inflammatory (Guo *et al.*, 2011), antipyretic activities, antioxidant and antimicrobial activities have been also proven (Karmakar *et al.*, 2015a). Cellular and nuclear damage activities and antitumor activities were also determined recently (Karmakar *et al.*, 2015b). Various phytopharmaceuticals like berberine, dictamnine, xanthoplanine, armatamid, asarinin and fargesin, alpha and beta-amyrins and lupeol are present in the plant (Kalia *et al.*, 2015; Nadkarni, 2002). The aim of the present study was structure elucidation of the isolate and its antioxidant and cytotoxic activity.

MATERIALS AND METHODS

Plant materials

Zanthoxylum alatum leaves were collected from the hilly region of Gangtok, Sikkim, India. The plant material was identified by the Botanical Survey of India, Howrah, West Bengal, India. A voucher specimen (CNH/38/2014/Tech. II/78) has been preserved in our laboratory for future reference.

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Extraction and Isolation

Dried powdered leaves (1.1 kg) were consecutively extracted by petroleum ether (7.4% w/w, yield), chloroform (9.1% w/w, yield) and methanol (10.6% w/w, yield) by using soxhlet apparatus. The solvents were completely removed under reduced pressure in a rotary vacuum evaporator (Buchi R-210). The concentrated extracts were stored in vacuum desiccators for further use. The isolation process was done for chloroform extract on column chromatography over silica gel (60-120 mesh). Hexane, ethyl acetate and methanol were eluted according to increase the polarity. An amorphous powder was found by eluted the solvent hexane: ethyleacetate (85:15) and characterized by ultra violet–visible (UV–vis), infrared spectroscopy (IR), mass spectrometry and proton and carbon-13 nuclear magnetic resonance (NMR).

***In vitro* antioxidant activity**

Antioxidant property of Zanthonitrile (1, 5, 10, 25, 50 µg/ml) was determined in triplicate by DPPH radical scavenging activity according to the standard method (Karmakar *et al.,* 2011). The 50% inhibitory concentration (IC_{50}) was calculated from graph as concentration versus percentage inhibition using the previously used formula.

***In vitro* cytotoxic activity**

Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India prior to the experiment and maintained in Swiss albino mice according to the standard protocol (Karmakar *et al.,* 2013). *In vitro* cytotoxicity of Zanthonitrile was determined using standard MTT assay with some modification (Nikhil *et al.,* 2014). In brief, EAC cells (3×10^5 ml^{-1}) were seeded into 96-well flat microtiter plates in enriched RPMI 1640 medium (200 µl) supplemented with penicillin (100 IU/ml) and streptomycin (100 µg/ml). Different concentrations Zanthonitrile (10, 25, 50, 100 µg/ml) was added to the cell in the volume of 100 µl/well. All samples were incubated for 24 h at 37 °C in a humidified incubator with 5% of CO_2. After 24 h, the medium was removed and cell cultures were incubated with 20 µl MTT reagent (5 mg/ml) for 4 h at 37 °C. DMSO (150 µl) was added for to remove the formazan produced by the viable cells. The suspension was placed on micro-vibrator for 5 min and absorbance was recorded at 570 nm by the ELISA reader. The experiment was performed in triplicate. The percentage cytotoxicity was calculated using the following formula.

The percentage inhibition was calculated as=

$$\frac{\text{100-[(Mean OD of treated cell}\times\text{100)}}{\text{mean OD of vehicle treated cells (negative control)]}}$$

The IC_{50} values were calculated using graph pad prism, version 5.02 software (Graph Pad Software Inc.,CA, USA).

Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.02, San Diego, California) Software. All data are expressed as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

The present study showed that treatment with the Zanthonitrile obtained from *Zanthoxylum alatum* leaves possesses antioxidant and cytotoxic activity. Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems.

Zanthonitrile (4-[{3'-methyl-2'-buten-1'-yl} oxy] phenyl acetonitrile) was isolated from *Zanthoxylum alatum* as a light yellow colored liquid. The ESI-MS spectral data showed molecular ion $[M]^+$ at *m/z* 201, indicating a molecular formula of $C_{13}H_{15}NO$. The IR spectrum revealed nitrile absorption at 2257.13cm^{-1}. The 1H NMR spectrum of isolated compound showed two singlates at 6.53ppm [(2H, d, *J*) 8.6 Hz, H-3 and H-5)] and 6.91 [(2H, d, *J*) 8.6 Hz, H-2 and H-6)] describing presence of a 1,4-disubstituted benzene. Furthermore, one substituent was proved to be a 3,3-dimethylallyloxyl group at 5.51ppm [(2H, d, *J*) 6.8 Hz, H-2'], 5.50 ppm [(1H, t, *J*) 6.8 Hz, H-2'], 1.72 and 1.76 ppm (each 3H, s, H-4' and H-5')], and acetonitrile CH_2- substituent proton was observed as a singlet at 3.68ppm (2H, H-1"). 13C NMR spectra confirmed the acetonitrile substituent through the peak at 23.1ppm (C-1") and a nitrile peak at 118.7 (C-2"). ESI-MS spectral data showed the presence of a prominent fragments at *m/z* 69 $[CH_2CHC(CH_3)_2]+$ and *m/z* 133$[HOC_6H_4CH_2CN]+$ indicates the presence of 2-methyl butene and 4-hydroxy phenyl acetonitrile indicates presence of 4-[{3'-methyl-2'-buten-1'-yl} oxy] phenyl acetonitrile which is named as zanthonitrile.

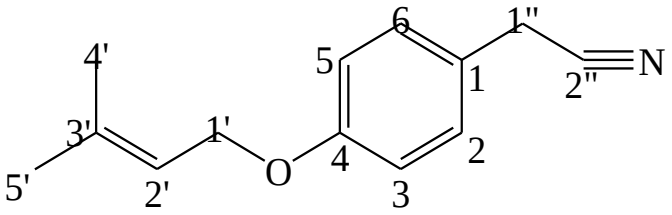

Fig. 1: Structure of Zanthonitrile.

Free radical is a molecule which has a single unpaired electron in an outer orbital. It may produce many diseases like cancer, arthritis and neurological disorder by the oxidative damage to the cells. The antioxidant effect on DPPH is believed to be due to their hydrogen-donating ability (Ashafa *et al.,* 2010). DPPH assay is the most widely reported *in vitro* method using for screening of antioxidant activity based on the reduction of colored free radical DPPH by free radical scavenger. DPPH gives violet color stable, nitrogen-centred free radical in methanol solution which was reduced to yellow colored diphenylpicryl hydrazine, with the addition of Zanthonitrile in a concentration-dependent manner (Karmakar *et al.,* 2011). The assay showed the inhibitory ability of Zanthonitrile and standard ascorbic acid on DPPH in a concentration dependent manner with the IC_{50} values 7.86 ± 0.23 µg/ml, and 9.17 ± 0.39 µg/ml (Fig. 2) which indicates the efficient DPPH scavenging activity.

In vitro cytotoxicity of Zanthonitrile was evaluated by the MTT reduction assay, after 24 h of exposure in culture. The

assay showed cytotoxic effect of Zanthonitrile on the EAC cell in a concentration dependent manner. The IC_{50} value was found to be 57.28 ± 0.64 µg/ml by plotting the graph concentration versus percentage inhibition (Fig. 3).

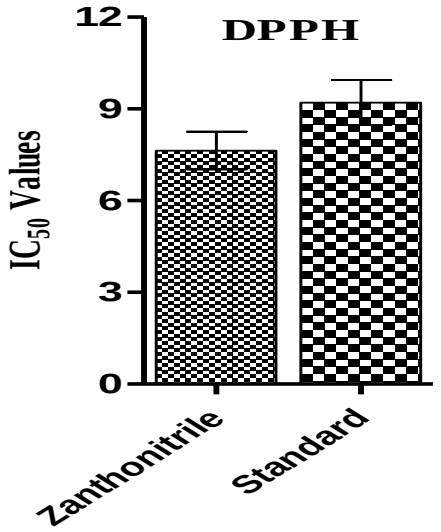

Fig. 2: The IC_{50} values of Zanthonitrile and standard for DPPH are 7.64 ± 0.62 µg/ml and 9.21 ± 0.74 µg/ml; The results are mean ± SEM of three experiments.

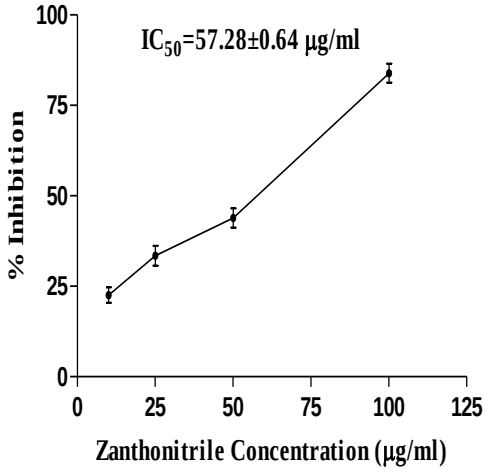

Fig. 3: Cytotoxic effect of Zanthonitrile on *in vitro* EAC cell. Values are mean ± S.E.M.; where n = 3.

The Ehrlich ascites carcinoma (EAC) cells are available as gray-white or light bloody viscose liquid. It referred to as rapid proliferation, undifferentiated carcinoma, shorter life span and 100% malignancy. EAC has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate (Ozaslan *et al.,* 2011). The cytotoxic effect of Zanthonitrile was investigated *in vitro* using MTT assay. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells (Zare Shahneh *et al.,* 2013). MTT results showed that Zanthonitrile possessed cytotoxic effect against EAC cell lines in a dose-dependent manner.

CONCLUSION

The isolate Zanthonitrile, proves to a potent antioxidant as is evidenced from the DPPH radical scavenging activity. It also has antitumor properties as is evinced from the *in vitro* cytotoxicity test and hence warrants further investigation of this compound in cancer research.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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REFERENCES

Ashafa AOT, Grierson DS, Afolayan AJ. *In vitro* antioxidant activity of extracts from the leaves of *Felicia muricata* Thunb. An underutilized medicinal plant in the eastern cape province, South Africa. Afr J Tradit Complement Altern Med, 2010; 7:296-302.

Geweli MB, Awale S. Aspects of Traditional Medicine in Nepal, Institute of Natural Medicine University of Toyama, Japan; 2008, pp. 140-142.

Guo T, Deng YX, Xie H, Yao CY, Cai CC, Pan SL, et al. Antinociceptive and anti-inflammatory activities of ethyl acetate fraction from *Zanthoxylum armatum* in mice. Fitoterapia, 2011; 82:347-351.

Jain N, Srivastava SK, Aggarwal KK, Ramesh S, Kumar S. Essential oil composition of *Zanthoxylum alatum* seeds from northern India. Flavour Frag, J 2001; 16:408–410.

Kalia N, Singh B, Sood R. A new amide from *Zanthoxylum armatum.* J Nat Prod, 1999; 62:311-312.

Karmakar I, Dolai N, Kumar RBS, Kar B, Roy SN., Haldar PK. Antitumor activity and antioxidant property of *Curcuma caesia* against Ehrlich's Ascites Carcinoma bearing mice. Pharm Biol, 2013; 51:753-759.

Karmakar I, Dolai N, Saha P, Sarkar N, Bala A, Halder PK. Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Orient Pharm Exp Med, 2011; 11: 221-228.

Karmakar I, Haldar S, Chakraborty M, Chaudhury K, Dewanjee S, Haldar P K. Regulation of apoptosis through bcl-2/bax proteins expression and DNA damage by *Zanthoxylum alatum*. Pharm Biol, 2015b; 54: 503-508.

Karmakar I, Haldar S, Chakraborty M, Dewanjee S, Haldar P K. Antioxidant and Cytotoxic Activity of Different Extracts of *Zanthoxylum Alatum.* Free Rad Antiox, 2015a; 5:21-28.

Nadkarni AK. Nadkarni's Indian Material Medica, vol. I Rep. ed. Popular Prakashan, Mumbai; 2002, pp. 569-570.

Nikhil K, Sharan S, Chakraborty A, Bodipati N, Krishna Peddinti R, Roy P. Role of isothiocyanate conjugate of pterostilbene on the inhibition of MCF-7 cell proliferation and tumor growth in Ehrlich ascitic cell induced tumor bearing mice. Exp Cell Res, 2014; 320 (2):311-328.

Ozaslan M, Karagoz ID, Kilic IH, Guldur ME. Ehrlich ascites carcinoma. Afr J Biotechnol, 2011; 10(13):2375-2378.

Ranawat L, Bhatt J, Patel J. Hepatoprotective activity of ethanolic extracts of bark of *Zanthoxylum armatum* DC in CCl_4 induced hepatic damage in rats. J Ethnopharmacol, 2010; 127:777-780.

Singh TP, Singh OM. Phytochemical and pharmacological profile of *Zanthoxylum armatum* DC. An overview. Indian J Nat Prod Resour, 2011; 2:275–285.

Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadav S. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. J Vector Borne Dis, 2007; 44:198-204.

Zare Shahneh F, Baradaran B, Orangi M, Zamani F. *In vitro* Cytotoxic Activity of Four Plants Used in Persian Traditional Medicine. Adv Pharm Bull, 2013; 3(2):453-455.

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