Int. J. Chem. Sci.: 7(1), 2009, 1-5

PRELIMINARY PHYTOCHEMICAL SCREENING AND STUDY OF ANTIVIRAL ACTIVITY AND CYTOTOXICITY OF *WRIGHTIA TINCTORIA*

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ABSTRACT

Wrightia tinctoria was investigated for the preliminary phytochemical analysis and characterization by various instrumental techniques. Indole derivatives such as isatin, induribine, tryphanthrine and fatty acids were identified. Methanolic extract of leaf parts of *Wrightia tinctoria* (WT) have been studied against replication of HCV in Huh 5.2 cells. The 50% effective concentration for inhibition of HCV in RNA subgenomic replicon replication in huh 5-2 cells (luciferase assay) by CWT was found to be 15 µg/mL. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells by 50% was greater than 50 µg /mL.

Key words : *Wrightia tinctoria,* Anti-HCV activity, Cytostatic

INTRODUCTION

Wrightia tinctoria (Syn. Pala indigo plant) of Apocyanaceae family is widely used in skin diseases, liver disorders and broad spectrum biological activities[1]. Viral hepatitis is a life threatening and debilitating disease state caused by Hepatitis-C virus (HCV). The *Wrightia tinctoria* flower has been reported to have a good anti-inflammatory activity[2]. Many compounds of plant origin have been identified that inhibit different stages in the replication cycle of virus[3-4]. *Wrightia tinctoria* is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases[5] and it was reported to possess analgesic[6] and cytotoxic[7] activities. Preliminary phytochemical screening and analytical investigations were performed to identify the chemical constituents. The present study was designed to screen the antiviral activity of methanolic extracts of *Wrightia*

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tinctoria (MWT) against the replication of Hepatitis C virus (HCV) RNA subgenomic replicon replication in huh 5.2 cells. The cytotoxicity of *Wrightia tinctoria* was also tested against mock-infected Huh 5.2 cells.

EXPERIMENTAL

Material and methods

Extraction: Fresh leaves of plant were collected from South Tamilnadu (Sankaran kovil) area in the month of September and were authenticated by a taxonomist from Pharmacognosy and Phytochemistry Department, AKCP, Krishnankovil, Tamilnadu. It was shade dried and subjected to maceration process. It was then defatted with petroleum ether and the residue was extracted with methanol, evaporated to dryness and stored.

Preliminary phytochemical screening : Phytochemical screening of the MWT was carried out for the presence of indole alkaloids, flavones and fatty acids.

Instrumental analysis

Ultraviolet spectroscopy : UV analysis of 5 mg/mL of the methanolic extract was performed from 200 to 800 nm for the determination of λ_{max}. Three maximum absorbance (λ_{max}) for the methanolic extract were observed by UV analysis.

Chromatographic techniques

Thin layer chromatography

20 µL of methanolic extract of *Wrightia tinctoria* was applied on precoated TLC plate. The plate was then developed in the following solvent systems. Solvent system I : Ethyl acetate : Hexane (40 : 60); Solvent system II : Chloroform : Methanol : Acetic acid (80 : 10 : 10); Solvent system III : Dichloromethane (100%) in a twin through chamber up to a distance of 8 cm. (Temp 25^0C – 30^0C RH : - 35% 40%). After development, the plate was dried and scanned at 540 nm. The resolved bands were identified.

High performance liquid chromatography

Preparation of sample solution : 5 mg of MWT extract was weighed accurately and the volume was made up to 1 mL using methanol. It was sonicated and centrifuged for 20 min. The supernatant of the solution was later subjected into HPLC.

Preparation of standard solution of marker compounds : About 5 mg of isatin and indirubine were weighed accurately and was made up to 1 mL with standard methanol and was then subjected to HPLC.

Detection of fatty acids using gas chromatography (GC)

5 mg of *Wrightia tinctoria* powder was macerated with hexane for 3 days. The solution was filtered. The filtrate was collected and extracted with methanol. The extracted methanolic solution was neutralized with sodium hydroxide and finally washed with water. The methanol layer was separated and then subjected to GC.

Anti-HCV activity of MWT by using replicon replication assay

Antiviral activity against HCV in Huh 5-3 cell : Replication assay undertaken with Huh-5-2 cells[16-19][a cell line with a persistent HCV replication 1389luc-ubi-neo/NS3-3/5.1; replication with firefly luciferase-lubquitin-neomycine phosphotransferase fusion protein EMCV-IRES drivan NS3-5B HCV polyprotein] was cultured in RPMI medium 2 mM glutamine, 1x non essential amino acid (Life Technologies, DC); 100 IU/mL penicillin and 100 µg/mL streptomycin and 250 µg/mL G418 (Geneticin, Life Technologies Washington DC). Cells were seeded at a density of 7000 cells per well in 96 well view plate TM (Packard, CA) in medium containing the same compounds as described above, except for G418. Cells were allowed to adhere and proliferate for 24 hr. At that time, culture was removed and serial dilution of test compounds were added in culture medium lacking G418. Interferon- α 2a (500 IU) was added as a positive control. Plates were further incubated at 37ºC and 5% CO_2 for 72 h. Replication of HCV replicon in Huh-5 cells results in luciferase activity in the cells.

Luciferase activity was measured by adding 50 µL of 1 x Gloysis buffer (Promega) for 15 min followed by adding 50 µL Steady-Glo Luciferase assay reagent (promega). Luciferase activity was measured with luminometer and signal in each individual well was expressed as a percentage of the untreated culture. Parallel culture of Huh 5-2 cells, seeded at a density of 7000 cells/well of classical 96-well cell culture plates (Becton-Dicknson) were treated in a similar fashion except that no Glo-lysis buffer or Stady-Glo Luciferase reagent was added. Instead the density of the culture was measured by means of the MTS method (Promega). The antiviral activity and cytotoxicity of the test compounds are showed in Table 1.

Table 1: Anti-HCV activity and cytotoxicity of *Wrightia tinctoria*

Compound	EC_{50} (µg/mL) HCV RNA*	EC_{50} (µg/mL) cell growth*	SI
MWT	**> 50**	**15**	**> 3**

*(% untreted control)
*Interferon α-2b at 10.000 units/well reduced the signal in the viral RNA (luciferase) assay to background levels; without any cytostatic activity.

RESULTS AND DISCUSSION

Herbal standardization of *Wrightia tinctoria* was performed and the following results were observed by using various chemical and instrumental analytical techniques. Preliminary phytochemical analysis of methanolic extract of *Wrightia tinctoria* showed the presence of alkaloids and flavones. The instrumental analysis of MWT was carried out using various analytical techniques such as UV, TLC and HPLC, which showed the presence of indole derivatives such as isatin and indurubine[5] (data are not shown). Fixed oils such as myristic acid, palmitoleic acid and behenic acid were identified by using GC (data are not shown). The maximum absorbance (λ max) of isatin and tryphanthrine were found to be 208 and 278, respectively. The 50% effective concentration for inhibition of HCV subgenomic replicon replication in huh 5-2 cells (luciferase assay) by MWT was observed as 15 µgm/mL. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells by 50% was greater than 50 µg /mL.

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Accepted : 01.11.2008