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ORIGINAL ARTICLE

Screening of ethnic medicinal plants of South India against influenza (H1N1) and their antioxidant activity

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Abstract Antiviral activity against H1N1 influenza was studied using ethnic medicinal plants of South India. Results revealed that *Wrightia tinctoria* (2.25 μg/ml) was one of the best antidotes against H1N1 virus in terms of inhibitory concentration of 50% (IC_{50}) whereas the control drug Oseltamivir showed 6.44 μg/ml. *Strychnos minor*, *Diotacanthus albiflorus* and *Cayratia pedata* showed low cytotoxicity (>100) to the MDCK (Malin darby canine kidney) cells by cytotoxicity concentration of 50% (CC_{50}) and possessed antiviral activity suggesting that these plants can be used as herbal capsules for H1N1 virus. *W. tinctoria* and *S. minor* showed high therapeutic indexes (TI) such as 12.67 and 21.97 suggesting that those plants can be used for anti-viral drug development. The CC_{50} values of *Eugenia singampattiana* (0.3 μg/ml), *Vitex altissima* (42 μg/ml), *Salacia oblonga* (7.32 μg/ml) and *Salacia reticulata* (7.36 μg/ml) resulted in cytotoxicity of the MDCK cells, due to their high phenolic content. Findings from this study state that the plant *W. tinctoria* can be a potent source for third generation anti-viral drug development against H1N1.

1. Introduction

Viruses are one of the major infective agents causing various health problems to humans resulting in death every year (Rajasekaran et al., 2013). Among the viral diseases influenza plays a vital role in humans and animals causing serious illness and major financial strain/stress. H1N1 viruses are more contagious and were reported to have caused serious problems in many countries. In Canada, the influenza virus was identified in 1 of 1.6 admissions during 2010 influenza season

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(Schanzer et al., 2013). This influenza can be divided into various subtypes like A and B and these two types of H1N1 virus were predominantly found among the human population affecting their day-to-day life. Generally these viruses cause acute respiratory infections referred to as "flu" resulting in serious problems particularly to children. Even though vaccines are available for flu, it was reported that only 50% were effective among the elderly persons (Wang et al., 2006). Moreover this virus subtypes A and B spread globally and its mutations that create antigenic drift and shift have been reported (Stein et al., 2009). This necessitated a serious search for better antiviral drugs.

Medicinal plants are termed to be one of the easy sources to get antiviral drugs since they have a proven record for antiviral activity. Tribals living worldwide traditionally have been using most of the medicinal plants successfully for many decades (Gurib-Fakim, 2006). Transmitting the medicinal plant formulae for curing some lethal diseases orally from generation to generation resulting in the loss of various valuable medicinal plant information (Nadembega et al., 2011). Even today a majority of the developing country's population depends on herbal medicines for their primary health care (Goleniowski et al., 2006). Due to this reason studies on medicinal plants to find new anti-viral drug developments for various diseases were required, since 40% of all chemical drugs were derived from the plant source. India is where Siddha and Ayurveda medicines are common and the Traditional healers, spread all over the country have immense knowledge in curing many human diseases by using medicinal plants (Muthu et al., 2006). Medicinal plants with a potential to cure can be taken up for scientific studies to see if it can combat viral diseases, with the hope of finding next generation drugs for influenza.

Coscinium fenestratum, *Trichopus zeylanicus*, *Eugenia singampattiana*, *Vitex altissima*, *Strychnos minor*, *Diotacanthus albiflorus*, *Strychnos nux-vomica*, *Chloroxylon swietenia*, *Helicteres isora*, *Andrographis paniculata*, *Wrightia tinctoria*, *Cayratia pedata*, *Salacia oblonga* and *Salacia reticulata* are some of the medicinal plants commonly used by natives and tribals of Tamil Nadu, South India. Among them *S. minor* and *E. singampattiana* were used by Kani tribes of South India for snake bites (Ayyanar, 2008), asthma and as anti-tumour agent(Viswanathan et al., 2006; Kala et al., 2011). *Coscinium* sps., and *Salacia* sps. were reported for their anti-diabetic and anti-inflammatory activities (Nayak et al., 2013; Ravishankar et al., 2013; Yoshino et al., 2009; Ismail et al., 1997). *Chloroxylon* sps., *Cayratia* sps. and *Trichopus* sps. were reported for their antioxidant activity and metabolic content by Nilip and Gouri, (2013), Perumal et al., (2012), Tharakan et al., (2005). Duraipandiyan et al., (2006), Ponnusamy et al. (2011) studied the antimicrobial properties of *Diotacanthus* sps. and *Wrightia* sps.

A. paniculata was reported for use against flu and possesses antiviral activity (Arora et al., 2010; Coon and Ernst, 2004). Apart from *A. paniculata* all other plants were reported for antioxidant properties and not against antiviral properties. Since these plants are highly used as medicine by the natives their activity against H1N1 influenza virus will provide alternative therapeutic formulation and be helpful for the human kind. Previous studies of herbal made medicines like Shahakusan, hochuekkito, Jinchai and Lianhuaqingwen capsules are proven for its effectiveness against virus by blocking transcription and replication resulting in the reduction of the illness period (Dan et al., 2013; Hokari et al., 2012; Zhong et al., 2013; Duan et al., 2011).

Based on this background, a screening of selected south Indian medicinal plants which are mostly used by ethnic and native people are tested against H1N1 influenza virus. This study will be helpful for new therapeutic agent (third generation influenza therapeutic compounds) preparation from the medicinal plant which can be helpful for the humans to overcome influenza since the virus was reported for its high mutation ability against drugs.

2. Materials and methods

2.1. Chemicals

All the solvents used for the study were HPLC grade and the chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Plant materials

C. fenestratum (MP1), *T. zeylanicus* (MP2), *E. singampattiana* (MP3), *V. altissima* (MP4), *S. minor* (MP5), *D. albiflorus* (MP6), *S. nux-vomica* (MP7), *C. swietenia* (MP8), *H. isora* (MP9), *A. paniculata* (MP10), *W. tinctoria* (MP11), *C. pedata* (MP12, rhizome), *C. pedata* (MP13, collected from Kanyakumari Dt, Tamil Nadu, India), *C. pedata* (MP14, collected from Kanchipuram Dt, Tamil Nadu, India), *S. oblonga* (MP15) and *S. reticulata* (MP16) plants were separately collected from tropical and western Ghats region of South India. All the collected plants were identified and confirmed by an ethno-botanist from Pachaiyappa's college, Chennai, Tamil Nadu, India. The leaves were shade dried before grinding and served as plant source for the extraction.

2.3. Extraction of medicinal plants

All the plant samples (6 replicates) were taken separately and weighed (0.1 g) in an Eppendorf tube (2 ml). 1 ml of 80% methanol was added to the samples and vortexed, followed by sonication for a period of 10 min. After that the methanol was collected separately by centrifugation at 8000 rpm. This step was continued twice and all the collected supernatants were added together and evaporated to dryness using speed vac. The resulting residues were redissolved in DMSO and used for cell line studies. In the mean time for the total phenolic and flavonoid analysis, these extracts were dissolved using 100% methanol.

2.4. Cell culture

Madin Darby canine kidney (MDCK) cell and influenza AP/R/8 virus (H1N1) were used for the present study. Influenza AP/R/8 virus and MDCK cells were purchased from American Type Culture Collection (ATCC). MDCK cell was maintained at 32 °C with 5% of CO_2 in a relative humidified cell culture incubator. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% of fetal Bovine Serum (FBS) and 1% of Antibiotic–Antimycotic solution (100×) was used for MDCK cell growth. DMEM, trypsin–EDTA, Antibiotic–Antimycotic Solution 100x and FBS were purchased from