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Research Article

DNA DAMAGE PROTECTION AND HAEMOLYTIC ACTIVITY OF ISOLONGIFOLENE

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ABSTRACT
In this study a Sesquiterpene, Isolongifolene of *Murraya koenigii* tested for its DNA protecting, genotoxicity and haemolytic activity. DNA Protecting activity was tested against genomic DNA using various concentrations of isolongifolene and the band intensity of each concentration was calculated. Genotoxicity was studied using pUC19 with different concentrations of isolongifolene and the activity was analysed using gel electrophoresis. Haemolytic activity was assayed using human erythrocytes and the activity was measured at 247 nm. Result shows that isolongifolene may protect the genomic DNA from radical damage and it does not produce any toxicity to the pUC19. The haemolytic activity isolongifolene shows the IC_{50} value of 50.42 which does not cause haemolysis. Hence, based on the results we conclude that isolongifolene has good protecting activity which can be used for further pharmaceutical studies.
Keywords: Isolongifolene; *Murraya koenigii;* DNA damage; genotoxicity; haemolytic activity

INTRODUCTION

In the normal cells, free radicals and reactive oxygen species (ROS) can be effectively eliminated by an enzyme-mediated system such as superoxide dismutase, peroxidase, glutathione peroxidase and non-enzymatic factors such as ascorbic acid, protein. When the homeostasis between the pro oxidant formation and antioxidant capacity is disrupted, whereby pro oxidant formation exceeds antioxidant capacity, oxidative damage will accumulate and result in patho-physiological events. Accumulating evidence indicates that active oxygen and free radicals would attack key biological molecules such as DNA, protein, and lipid that leading to many degenerative disease conditions[1]. The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti inflammatory, anti necrotic, neuro protective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity[2,3]. *Murraya koenigii* (Linn) Spreng is a tropical tree of the family Rutaceae, which is native to India. The leaves used as herb in Ayurvedic medicine. Their properties include much value as an anti-diabetic[4-7], antioxidant, antimicrobial[8-11], anti inflammatory[12], hepatoprotective[13], anti hypercholesterolemic[14,15]; as well as efficient against colon carcinogenesis. So far, thirteen compounds were identified in *Murraya Koenigii* leaves. Isolongifolene present in *Murraya Koenigii* leaves and it has anti-proliferative activity. The aim of this work was to study the DNA protecting and Haemolytic activity.

MATERIALS AND METHODS

Isolongifolene, pUC19, H_2O_2, tritonX, agarose were purchased from sigma Aldrich.

DNA Nicking Assay

DNA nicking assay was performed using genomic DNA by the method of lee *et al*., 2002[16]. Different concentrations ranging from 2-6 mg of isolongifolene was taken in a microfuge tube. To this 10 µl of fenton's reagent (30 mM H_2O_2, 50 µM Acetic acid and 80 µM $FeCl_3$) was added and incubated for 30 minutes at 37°C. The DNA was analyzed on 0.8 % agarose gel and it was viewed under UV illuminator.

Genotoxicity assay

Plasmid nicking assay was carried out by the method described by kitts *et al*., 2000[17]. A mixture of 10 µl (2 mg) of isolongifolene (sigma) and pUC19 (0.5 µg) was taken in a microfuge tube and incubated for 2 h pUC19 (0.5 µg) with 215 µM of H_2O_2 were served as negative control. 10 µl of the tracking dye was added to the tube (40 mM EDTA, 0.05 % bromophenol blue and 50 % (V/V) glycerol). After incubation the mixture was subjected to electrophoresis. The reaction mixture was run on 0.8 % agarose gel for 40 minutes. Ethidium bromide stained gel was viewed and photographed with UV illuminator. Finally the densitometry calculation was made with Gel Quant. NET software.

Haemolytic assay

Haemolytic activity was evaluated as described previously by Andra *et al*., 2008[18]. Human erythrocytes washed with Phosphate Buffer Saline (pH 7.4) and centrifuged at 8000 g for 10 minutes. After washing with PBS (until the supernatant was colourless), then the erythrocytes were re-suspended and diluted to 10 times of the original volume with PBS kept as stock. Then, 150 µl of isolongifolene (2-16 µg/ml) in PBS was incubated with 150 µl of stock erythrocyte suspension (4 % v/v) for 60 minutes at 37°C. After the incubation period, the reaction mixtures were centrifuged at 1,000 x g for 10 minutes to remove intact erythrocytes. The 10 fold dilution of the supernatant of released haemoglobin was measured at 540 nm using a micro plate reader. The triplicates experiment was done. Finally the haemolytic activity was expressed as a percentage haemolysis using the following equation. Finally the IC_{50} Value was calculated by using PRISM version 5 software.

% of Haemolysis = (Abs sample-Abs buffer/Abs maximum-Abs buffer) × 100

RESULTS AND DISCUSSION

Hydroxyl radical is one of the ROS formed in biological systems, causing DNA strand breakage, which leads carcinogenesis, mutagenesis and cytotoxicity[19].

DNA protective activity of isolongifolene was assessed on hydroxyl radical generated DNA stand breakage was

evaluated using genomic DNA. Different concentration of isolongifolene was treated with fenton's reagent and the protecting activity was shown Figure 1. Band intensity was calculated to analyze the effectiveness of isolongifolene and the result shown in graph 1. The results proved that isolongifolene protects DNA from damage caused by hydroxyl radicals and band intensity revealed that isolongifolene exhibits DNA protecting activity. Genotoxicity of isolongifolene was tested by the method described by kitts *et al*., 2000. The genotoxicity effect of isolongifolene was evaluated with pUC19 plasmid DNA. The genotoxicity effect of isolongifolene and band intensity was shown Figure 2 and graph 2. The plasmid DNA was mainly of super coiled, open circular and linear DNA and isolongifolene results that it does not affect any form of the plasmid DNA. Some of the Plant derived compounds are toxic to the cell. Sesquiterpene lactones are used to treat some diseases which may lyse heme. Isolongifolene was tested for its haemolytic activity. Haemolytic activity of isolongifolene was shown in graph 3. When compared to other plant derived compound saponins the IC_{50} value of isolongifolene was found to be 50.42. The results proved that isolongifolene possess insignificant to haemolysis.

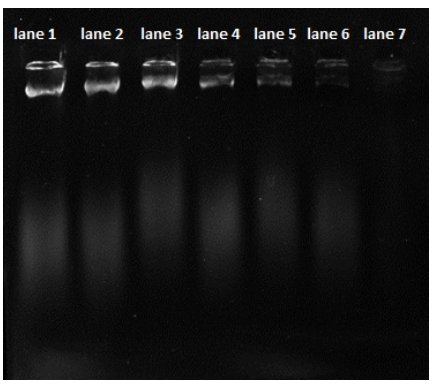
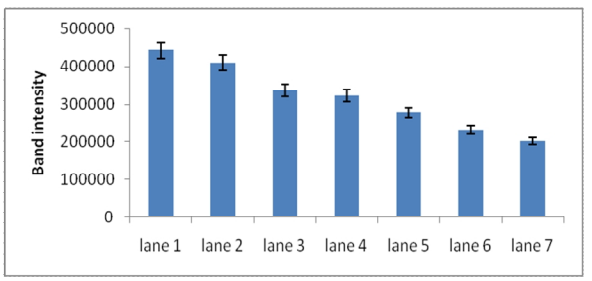


Figure 1: DNA protecting activity of Isolongifolene

Lane 1 - control Genomic DNA
Lane 2 - Fenton's reagent + Genomic DNA + quercetin
Lane 3 - Fenton's reagent + Genomic DNA + Isolongifolene (8 mg);
Lane 4 - Fenton's reagent + genomic DNA + Isolongifolene (6 mg)
Lane 5 - Fenton's reagent + Genomic DNA + Isolongifolene (4 mg)
Lane 6 - Fenton's reagent + Genomic DNA + Isolongifolene (2 mg)
Lane 7- Fenton's reagent + Genomic DNA



Graph 1: Band Intensity graph of DNA protecting activity

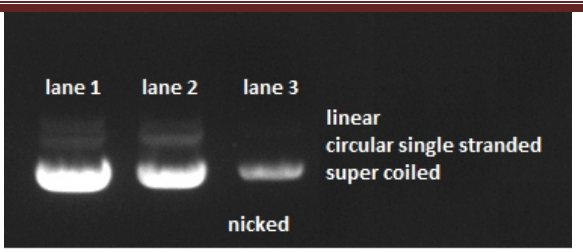
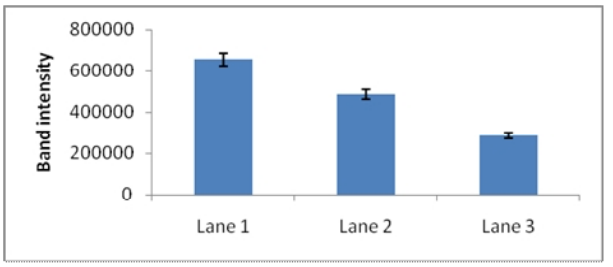
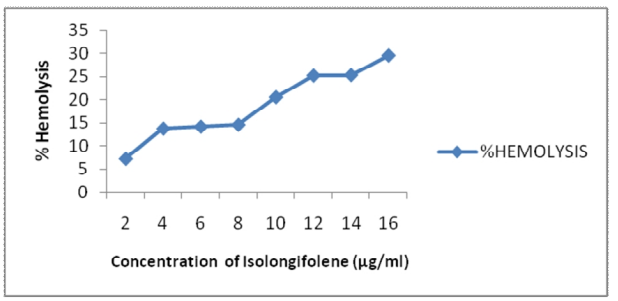


Figure 2: Genotoxicity activity of isolongifolene

Lane 1: control pUC19 plasmid DNA
Lane 2: pUC19 Plasmid DNA + 2 mg of isolongifolene
Lane 3: pUC19 Plasmid DNA + H_2O_2



Graph 2: Band Intensity graph of genotoxicity



Graph 3: Haemolytic activity of Isolongifolene

CONCLUSION

Isolongifolene does not cause damage, toxicity to DNA and it may not lyse the heme. The results conclude that isolongifolene has good protecting activity. Hence isolongifolene is safe for use and it can be further studied for its activity.

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