Antioxidant and Cytotoxicity Properties of Emodin- An Invitro Study

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Abstract

Introduction: Emodin which is a medicinal herb has shown to have various medicinal properties. The antibacterial efficacy of the Emodin extract was high in oral pathogens. Hence this study aims to determine the antioxidant and cytotoxicity properties of Emodin extract. **Methodology:**The cytotoxicity property was determined using the Brine Shrimp Lethality Assay whereras the antioxidant property was examined using the DPPH scavenging Assay. **Results:**The cytotoxic potential of the emodin extract was 60% in 200 microliter concentration, wheras the antioxidant potential increased with increasing concentration **Conclusion:**Emodin extract had a high cytotoxicity and antioxidant potential. This has a future scope of the study with testing this extract in the cancer cell lines.

Keywords Emodin, Antioxidant, DPPH, Cytotoxicity.

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INTRODUCTION

Emodin, an anthroquinone which is derived from the roots of Polygonum cuspidatum has various medicinal properties[1]. In relation to oral environment, Emodin has shown good anti-bacterial efficacy against oral micro-organisms which are the most important cause of oral infections[2]. Oral cancer is the sixth most frequent leading cause of cancer death worldwide; the early detection of malignant events is a high priority for reducing deaths due to oral cancer[3]. Oral squamous cell carcinoma is the most frequent form (>90%) of oral cancer, with 5-year survival rate of 50% despite various management in the past 3 decades. Oral leukoplakia is the most common oral potentially malignant lesion, with a higher tendency of malignant transformation[4].

Free radicals also known as Reactive Oxygen Species are unstable molecules generated by all cells in day to day

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biological processes [5]. However, uncontrolled production of these molecules can have a deleterious impact on cellular operations like cell membrane fluidity, permeability and ion channels activity[6]. The major threatening factor is that these free radicals form the basis for DNA damage causing mutagenic changes and cellular death. and their derivatives[7]. This is majorly responsible for disparity in redox homeostasis. It is also one of the prime factors leading to the development of chronic disorders, diabetes mellitus, coronary heart ailment, ageing and cancer[8]. Herbal dentistry is the newly exploring field as there are no after effects hence herbal medicines are used[9]. Emodin which is extracted from the roots of Polygonum cuspidatum has shown to have antibacterial property. It has been reported that emodin exhibits a wide range of biological activities including antibacterial, anticancer, anti-inflammatory, anti-diabetic and anti-oxidative activities. Emodin has been demonstrated to possess a wide spectrum of pharmacological effects, such as antiviral, antibacterial, anti-allergic, anti-osteoporotic, anti-diabetic, immunosuppressive, neuro-protective and hepatoprotective activities[10]. Moreover, the laxative property of emodin has been its primary mechanism of action for promoting weight loss.. Hence this study aims to find out the cytotoxicity and antioxidant potential as it can contribute in being a medication for pre malignant lesion without side effects.

Hence this study aims to determine the antioxidant and cytotoxicity properties of Emodin from Polygonum cuspidatum.

MATERIALS AND METHODS

This in vitro study was conducted in the Nanomedicine Lab, Saveetha Dental College, Chennai.

Cytotoxic Effects Brine Shrimp Lethality Assay

It is a general bioassay that is capable of detecting a broad spectrum of bioactivity in crude extracts. The commercial availability of cost efficient brine shrimp eggs, the safety and the ease of handling the assay, as well as no exceptional technological specifications makes this an efficient tool for the phytochemistry laboratory. This assay has been predominantly used to biomonitor the isolation of plant cytotoxic compounds.

Preparation Of Emodin Extracts

Emodin powder was obtained from online retailers of Chennai. Emodin extract was formulated by adding 0.5mg of emodin powder to 5ml of distilled water. The solution was then filtered using What man filter paper and the clear extract was then transferred to an airtight container.

Hatching The Shrimp

The brine shrimp eggs were hatched in artificial sea water prepared through dissolving 0.5 g of sea salt in 150ml of distilled water. After 24 hours of incubation at room temperature (27-29 degree celsius), the larvae (nauplii) were attracted to one side of the artemia tank with a light source and collected with pipette.6 tubes were taken that were filled with artificial sea water. 10 nauplli's were added to each of the tubes. Emodin was loaded in the concentration range of 10µl, 20µl, 30µl, 40µl, 50µl. A control tube was also prepared by adding 3ml of artificial sea water and 10 nauplii's. The tubes were kept for 24 hours incubation. After the incubation, the tubes were observed using a magnifying glass. The live and dead nauplii's were counted and percentage death was calculated.

Percentage death= Number of dead nauplii X 100/Number of dead nauplii-Number of live nauplii

Antioxidant Activity

Scavenging Assay was executed to monitor the antioxidant potential of plant crude

extract. DPPH is a lipophilic free radical, nitrogen centered with purple colour. The antioxidant donates an electron to DPPH radical there by colour changes to pale yellow gradually.

Test group: 10µl, 20µl, 30µl, 40µl, 50µl was taken in 5 test tubes respectively. To each tube,1ml of DPPH (2,2-diphenyl-1-picryl-hydrazyl) was added. 1990µl, 1980µl, 1970µl, 1960µl, 1950µl of 50% methanol solution was added to the test tube containing 10ul, 20ul, 30ul, 40ul, 50ul of emodin respectively.

Control group: 1ml of DPPH was added to 2ml of methanol solution. Standard group: Ascorbic acid was used as standard.

The test tubes were incubated in a dark cupboard for around 20 minutes. Absorbance was measured at 517nm in UV Spectrophotometer.

% inhibition was calculated using following formula:

% of inhibition=Control Absorbance-Sample Absorbance X 100/Control Absorbance

RESULTS

Table 1 depicts the cytotoxicity of emodin extract. The results show that there is 60% death of nauplii eggs at 200 µl. Hence the study affirms that emodin exhibits highest cytotoxicity at 200µl where as moderate cytotoxicity at 5µl and 25µl. There was a significant increase in percentage of death with an increase in the concentration. The maximum percentage of death was 60% noted at both 200µl. The involvement of free radicals, especially their increased production, appears to be a feature of most of human diseases including cardiovascular diseases and cancer. In this regard, the antioxidant activity of synthesized emodin extract was assessed by DPPH scavenging assays. The DPPH free radical is stabilized when it accepts electrons. The DPPH radical is purple in colour with a maximum absorbance at 517 nm. The observed results in the DPPH assay show free radical inhibition by emodin. Fig1. signifies the antioxidant activity of emodin extract. The values of antioxidant property of emodin are lower than the standard values at all concentrations. However, the antioxidant property of emodin increases with an increase in the concentration. The percentage of inhibition was 21% at 5µl, 37% at 25µl, 51% at 50µl, 57% at 100µl and 73% at 200µl.

Table 1: Cytotoxicity of Emodin extract

Concentration	5 microlitre	25 microlitre	50 microlitre	100 microlitre	200 microlitre	Control
% of lethality	30%	50%	50%	50%	60%	0

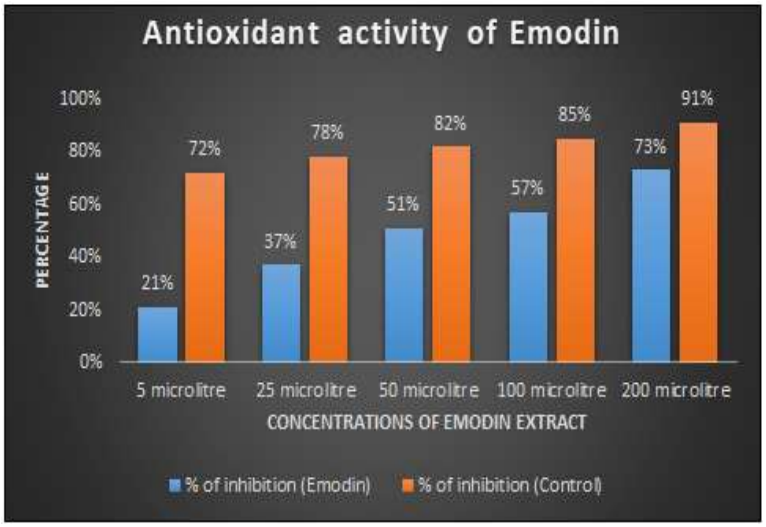


Figure 2: Antioxidant activity of Emodin extract

DISCUSSION

Cancer is a major threat to the mankind as suitable remedy is not available till date. Hence the medicinal activity of herbal plants are concentrated as it can provide sufficient treatment to eradicate the carcinogenic activity. Our study significantly had 60% cytotoxic effect at 200 microliter concentration. Antioxidant potential increased towards the higher concentration of emodin extract. Previous study suggested that crude extract of leguminosae species had a very high potential of cytotoxicity which is in accordance with the results of the present study. The antioxidant potential of these species were also high, but 23 plant species were used in this study and it was not identified which species had the highest cytotoxicity and antioxidant potential [11].

In a study conducted by Saurav et al, the Streptomyces species was cytotoxic to cancer cells and possesses antioxidant property. There was a Systematic screening of isolates for anti-Aspergillus activity resulted in the identification of *Streptomyces* species in this study [12]. In a study conducted with Lentinus crinitus which is a wild fungus, there was no cytotoxic effect but had a very high antioxidant potential [13]. In a study examining Cassia augustifolia flowers, the percentage of antioxidant activity was increased with increasing concentration of AgNPs. In addition, cytotoxic activity of the AgNPs was evaluated in human breast cancer cells (MCF 7). Phyto-synthesized AgNPs showed dose-depended manner of cytotoxic activity against MCF 7 cancer cells [14].

Hence this Emodin extract showed a high potential to antioxidant and cytotoxicity properties. The future scope of the study aims to test this component in the cancer cell lines as to accurately predict the cytotoxicity and antioxidant potential of this extract.

CONCLUSION

Emodin extract has a high potential to cytotoxicity when tested in-vitro and the antioxidant level of the emodin extract increases with increasing concentration.

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