

Antioxidant and antiproliferative activity of film based sodium alginate with *Cocculus hirsutus* mucilage

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ABSTRACT

Natural polymers are readily available and biodegradable; primarily they are commercial, their usage increased in food and pharmaceutical industry. Among them, sodium alginate is one of the most abundant renewable polymers used in the pharmaceutical, agricultural and chemical industries mainly due to its unique properties including antioxidant and antimicrobial properties, film-forming ability, biodegradability, biocompatibility, availability and non-toxicity. In recent years, plant derived polymers like mucilage have a great interest due to their wide range of applications such as thickening, binding, disintegrating, suspending, emulsifying, stabilizing and gelling agents. Mucilaginous material is soluble fiber rich compound so that it has a high-water holding capacity and shows similar functional properties to those of gum Arabic. The current study to evaluate antioxidant, antiproliferative activities of the different concentration of *Cocculus hirsutus* leaves mucilage incorporated into sodium alginate film. The most suitable properties of antioxidant, antiproliferative properties were determined by DPPH assay and MTT Assay. Antiproliferative activity was assessed using breast cancer cell line (MCF7), while the mucilage extract of *Cocculus hirsutus* showed the inhibition effects of the cancer cell lines (MCF7), while there was no effect on the growth of normal cells. So, the natural mucilage and their constituents incorporated into sodium alginate-based films as a promising technology with the knowledge that these compounds have been able to prevent oxidation and control the proliferation of cancer cell growth.

Keywords: Sodium alginate, *Cocculus hirsutus*, Antiproliferative activity and Antioxidant activity.

1. INTRODUCTION

Natural polymers comprise with a large number of the derivatives group, it can be exploited to its best since its readily available, can withstand chemical modification and due to its origin, it has a good potential to degrade. They are hydrophilic polymers which is very low-cost effective has positive regulatory acceptance for large scale production. Polymers consists of various characteristics which makes them an efficient choice for the versatility, a wide range of physical and chemical properties, non-toxic and have good mechanical strength, inexpensive and easy to construct, inert to host tissue and compatible with the environment. Composites are generally formed by physical interaction between two different polymer solutions. This type of

composites exhibits unique physical and chemical properties, as the interactions within the polymer gels have considerable effect on the formation and their properties, because it depends on various factors including nature and position of the ionic groups, molecular weight, charge density and concentration of both polymers.

Natural polysaccharides are excellent ingredients of both edible films and coatings. Among them, sodium alginate is one of the most abundant renewable polymers used in the medical field, food, agricultural and chemical industries mainly due to its unique properties including intrinsic wound-healing, antioxidant and antimicrobial properties, hemostatic activity, film-forming ability, biodegradability, biocompatibility and non-toxic effects.^[1,2] Among the natural plant

based polymers, mucilage are biocompatible, cheap and easily available and are preferred to semi synthetic and synthetic formulations because of their lack of toxicity, low cost, availability, emollient and non-irritant nature.^[3]

Plant mucilage are pharmaceutically important polysaccharides with a wide range of applications such as thickening, binding, disintegrating, suspending, emulsifying, stabilizing and gelling agents.^[4] They have also been used as matrices for sustained and controlled release of drugs. Mucilage is a complex polysaccharide, formed from sugar and uronic acid units and it form slimy masses in water, and are typically heterogeneous in composition.^[5] Upon hydrolysis the mucilage yields arabinose, galactose, glucose, mannose, xylose and various uronic acids. Mucilage's are obtained mainly from seeds or other plant parts. Some are obtained from marine algae, and from selected microorganisms. Some of the mucilage rich plants are Aloe vera, *Abelmoschus esculentus*, *Trigonella foenum-graceum*, *Asparagus racemosus*, *Lepidium sativum*, *Salvia hispanica*, *Althaea officinalis*, *Hibiscus rosa-sinensis* and *Cocculus hirsutus*. These are most commonly used as an adjuvant in pharmaceutical preparations ⁽⁶⁾. Mucilage has been found to play a role in water storage and retention, lubrication, seed germination and dispersal and also can function as a secondary reserve. In pharma industries, mucilage are used in medicine for their demulcent properties for cough suppression, ingredients of dental and other adhesives and can be used as bulk laxatives. These hydrophilic polymers are useful as a tablet binder, disintegrants, emulsifiers, suspending agents, gelling agents, stabilizing agents, thickening agents, film forming agents in transdermal and periodontal films, buccal tablets etc., They have also been used as matrices for sustained and controlled release drugs^[7].

The leaves of *Cocculus hirsutus* contain a high proportion of mucilage with polysaccharides and gelatinous type of materials. The majority of the traditional uses of leaves can be attributed to the mucilage content only. Mucilage is not absorbed in the G.I. tract and passes through the system undigested. *Cocculus hirsutus* leaves are used topically as emollient and demulcent. It has been non-toxic to human skin. *Cocculus hirsutus* is belonging to family Menispermaceae. It consists of 20 species of mostly scandent or rarely suberect herbs or shrubs (Santapau & Henry, 1976). It is widely distributed to Sudan, central Asia, China and India (throughout tropical and subtropical regions). The plant grows all over India, especially in dry regions. In India, it is known by various

names in different regions (Kirtikar & Basu, 2005).

Alginate is an important source for food industry, the rest being used in industrial, pharmaceutical and dental applications.^[8] Alginate is a hydrophilic polysaccharide extracted from marine brown algae such as *Laminaria hyperborea* or soil bacteria such as *Azotobacter vinelandii* and composed of 1,4-linked β -D-Mannuronic acid (M) residues and 1,4-linked α -L-guluronic acid (G) in various proportions, displaying carboxylic acid functionality at the C5 residue. ^[9]

Alginate is an effective natural disintegrant tablet, binder and offers an attractive alternative for sustained-release systems. It offers advantages over synthetic polymers as it forms hydrogels under relatively mild pH and temperature and is generally regarded as non-toxic, biocompatible, biodegradable, less expensive and abundantly available in nature.^[10] In addition, alginate meets the important requirement of being acquiescent to sterilization and storage. All these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and other biologically active compounds and also for the encapsulation of cells. It has been much used in medical applications such as wound dressings, scaffolds for hepatocyte culture and surgical or dental impression materials. Which are also known to be broken down to simpler glucose type residues and can be totally absorbed, and successfully used as a matrix for the entrapment and/or delivery of biological agents, such as drugs and proteins. ^[11,12]

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant

The plant *Cocculus hirsutus* was collected from the region of Cuddalore district and identified by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The leaves were collected from the plant and shade dried at room temperature. After that, it was powdered and used for further studies.

2.2. Extraction of Mucilage of *Cocculus hirsutus*

5 gm of leaves powdered *Cocculus hirsutus* were soaked in water for 5-6 hours, boiled for 30 minutes and kept aside for 1 hour for complete release of the mucilage into the water and filtered through eight-fold muslin cloth bags. The solvent ethanol was used for the maceration and subsequent precipitation. The mucilage was then dried by keeping the Petri dishes in an oven at 50°C. The dried mucilage powder was scraped out and grounded using a mortar and pestle.

Grounded powder was weighed and stored in desiccators for further use.

2.3. Preparation of Sodium Alginate Films

A solution of sodium alginate was prepared by dissolving 2.0g of sodium alginate and 0.01g of calcium chloride in 100 mL of sterile deionizer water. The solution was heated on a hot plate with stirring at 80 °C until it was completely dissolved all the solid content. After that the prepared mucilage solution was added, stirred at 1000 rpm for 25 min at room temperature until the solution became clear. The resulting solutions ALG-MU were poured on polythene trays having measurements 10 × 10 cm, and dried at room temperature for 48 hrs.



Figure -1: ALG-CH Mucilage.

2.4 Antioxidant activity

2.4.1. Determination of DPPH scavenging assay

DPPH radical scavenging activity of mucilage was determined according to the standard protocol. An aliquot of 0.5mL of mucilage solution was mixed with 0.5mL of methanol and to this 2.5mL of DPPH reagent was added. The mixture was shaken vigorously and incubated for 30min in the dark at room temperature. The absorbance was measured at 517nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging activity% was calculated using the formula.

$\% \text{ of inhibition} = \frac{\text{absorbance of control (A0)} - \text{Absorbance of Sample (As)}}{\text{Absorbance of control}} \times 100$. [13]

2.5. Cytotoxic activity

2.5.1. Cell culture and MTT assay

The Breast cancer cell line (MCF-7) were plated separately using 96 well plates with the concentration of 1×10^4 cells/well in EMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO₂ incubator at 37°C with 5% CO₂. The cells were washed with 200 µL of 1X PBS, then the cells were treated with various test concentrated compound in serum free media and incubated for 24 h. The medium was aspirated from cells at the end of the

treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37°C for 4 h using CO₂ incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of DMSO and thoroughly mixed. The development of color intensity was evaluated at 570nm. The formazan dye turns to purple blue color. The absorbance was measured at 570 nm using microplate reader. [14]

3. RESULTS

3.1. Antioxidant activity- DPPH Assay

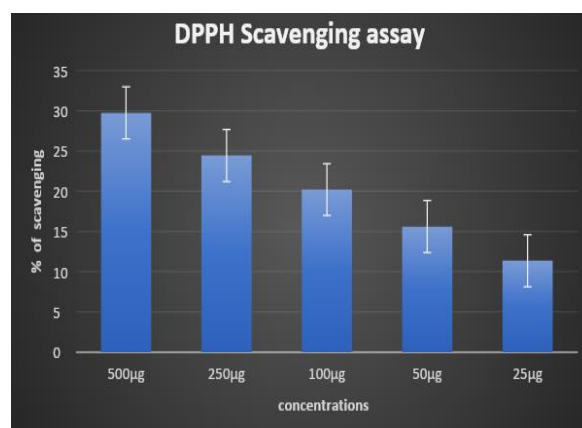


Figure - 2: Determination of antioxidant activity of DPPH scavenging activity of AL-MU.

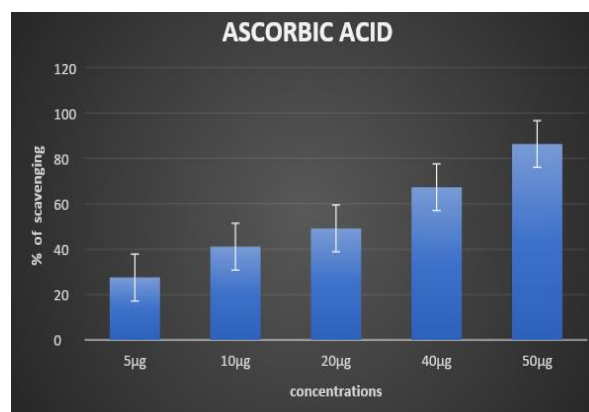


Figure - 3: Determination of antioxidant activity of DPPH scavenging activity of Ascorbic acid.

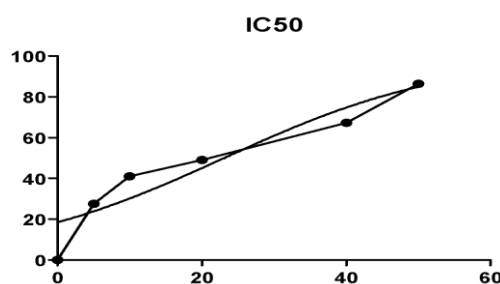


Figure - 4: IC₅₀ = 23.11 ± 2.05 µg

3.2. Cytotoxic activity

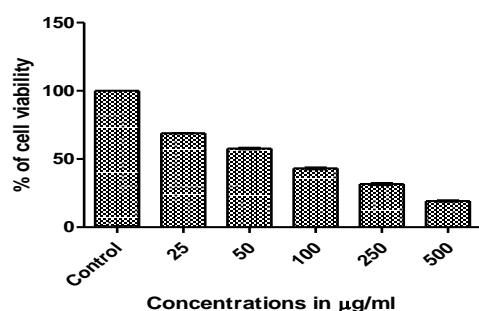


Figure - 5: Percentage of Cell viability in Breast cancer cell line

3.2.1. MTT Assay:

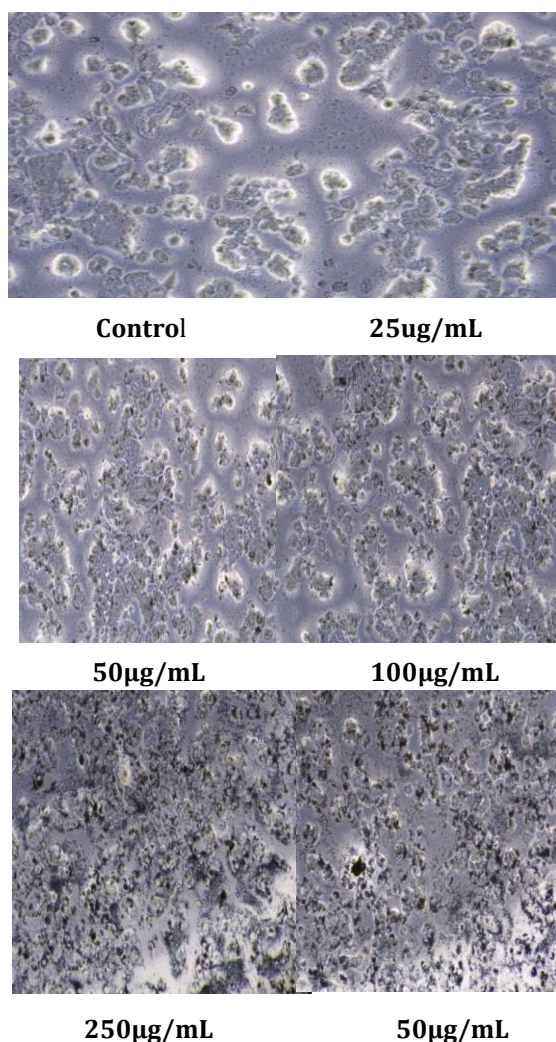


Figure - 6: Cell Viability ofALG-CH Mucilage at Various Concentration.

4. DISCUSSION

Mucilage’s are most commonly used as an adjuvant in pharmaceutical preparations with a wide range of applications such as thickening, binding, disintegrating, suspending, emulsifying, stabilizing and gelling agents. They have also been

used as matrices for sustained and control of releasing drugs. *Cocculus hirsutus* leaves contains a high proportion of mucilage. The majority of the traditional uses of leaves can be attributed to the mucilage content only.

Alginate is a non-toxic, biocompatible, biodegradable, less expensive and abundantly available in nature, all these advantages make alginates very useful materials for biomedical applications, especially for controlled delivery of drugs and other biologically active compounds and for the encapsulation of cells. Sodium alginate having the most suitable properties, different concentrations of 0-4% (v/v) of *Cocculus Hirsutus* mucilage was incorporated into sodium alginate film.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Plant-derived polymers contain different phenolic compounds including flavonoids and polyphenols as bioactive compounds. These bioactive compounds prevent the chain propagation reactions originated by free radical reactions and prevent disease-related oxidative damage. The most important assay for antioxidant screening is DPPH methods for plant extracts. DPPH produced violet color then reduced to a yellow-colored product; which is more stable; the addition of the fractions depends on its concentration. The results showed that the potential antioxidant effect at all the concentrations of mucilage compared with ascorbic acid (control). Sardarodiy an et al. also studied the antioxidant activity and total phenolic compounds of *Lallemantiaroyleana* seed mucilage and in their study, they observed antioxidant activity of 528.54 g/mL of DPPH.^[15]

Now a day’s lot of Carcinogenic compounds caused different types of Cancer. This disease caused by uncontrolled proliferation of cells in the human body. This situation the tumors may be formed from malignant cells with the potential to be metastatic. Recently handled treatments include chemotherapy, radiation and chemically modified drugs ^[16]. The patients put in to the treatments such as chemotherapy can face so many problems and also get further injury their

health. Therefore, researchers focus on using alternative medicines and therapies against cancer.

Antiproliferative activity shows in ALG-CHM film exhibits significant % of inhibition in the cell viability range. MTT assay basis to the anticancer studies, this procedure was followed to eradicate the in-vitro human cell line to measure the cytotoxic effect caused by carcinogenic substances by using MCF7(breast cancer). The ALG-CHM film showed that reduce the growth of cancerous cells and with the concentration of 25µg/mL, 50µg/ml, 100µg/mL, 250µg/mL and 500 µg/mL were used.. The result of ALG-CHM film effectively involved in cancerous cell to restrict significantly. The results suggested that ALG-CHM film inhibited the growth of MCF7 breast cancer cell in a very effective manner. Thus, the observed antiproliferative effect may be attributed to the presence of phytochemicals present in the *Cocculus hirsutus* leave smucilage.

5. CONCLUSION

In the present study, natural polymers of ALG-CH Mucilage film were assessed for its pharmacologic activity and it is concluded from this study that the sodium alginate with *Cocculus hirsutus* mucilage composite have good antioxidant property and antiproliferative activity against breast cancer MCF7 cell lines. The activity was found due to the presence of phytochemicals and Phytosterols present in the mucilage. Hence sodium alginate with *Cocculus hirsutus* mucilage film can be considered as a herbal drug for the treatment of free radical induced disorders like cancer, cardiac and many other life style diseases.

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