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MICROWAVE ASSISTED ISOLATION AND EVALUATION OF CHLOROPHYLL FROM WHEATGRASS AND STUDY ON ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Wheatgrass (Triticum aestivum) is one of the important herb consumed widely and is known for therapeutic and nutraceutical potential. The current work was focused to isolate and characterize chlorophyll from wheatgrass. The results demonstrated effective isolation of chlorophyll from wheatgrass. Thus, it could be concluded that microwave assisted extraction is one of the easiest, effective and reproducible method for extraction for chlorophyll. The study has also established antimicrobial potential of chlorophyll isolated from wheatgrass.

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INTRODUCTION

Chlorophyll is a green pigment found in chloroplasts of plants. Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy from light [1]. Therapeutically, chlorophyll is used in the treatment of chronic pancreatitis [2] and microbial infections [3].

Wheat grass from *Triticum aestivum* is one of the important gift of nature given to mankind. The scientific outlook was attracted to it when Schnabel, a food scientist demonstrated health benefit potential of wheatgrass on hens and chicks [4]. Today a number of research on wheatgrass demonstrates its anticancer [5, 6], antiulcer [7], wound healing [8], antioxidant [9] potential. Studies also demonstrate nutraceutical potential of wheat grass as it is rich in proteins, vitamins and minerals [10].

Microwave assisted extraction is one of the newer technique whereby less solvent consumption, less work time, good results, high recoveries and reproducibility in result is observed. Microwave assisted cooking is one of the effective way of cooking characterized by short cooking time, for example, spinach, when cooked in microwave retains all folate contents compared to stove [11-16].

The objectives of this work were to isolate chloroplasts from wheat grass juice by using microwave method by using micro-wave oven and to determine its antimicrobial activity on selected pathogens.

EXPERIMENTAL

Chemicals

Until otherwise specified all the chemicals were purchased from CDH, India.

Test organisms

Bacterial and fungal cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The microbial strains were *Escherichia coil* MTCC 2960, *Pseudomonas auruginosssa* MTCC 4676, *Staphylococcus aureus* MTCC 3160, *Klebsiella oxytoca* MTCC 3030, *Bacillus subtilis* MTCC 1790, *Candida albicans* MTCC 183.

Analytical measurements

IR spectra were recorded on a Shimadzu FTIR spectrometer. The UV-Vis spectra were recorded on a Shimadzu spectrophotometer (200-900 nm) in 80% aqueous acetone solution.

Plant collection and extraction

Wheat grass from *Tritium aestivum* was grown in our laboratory. A nine to eleven day matured leaflings were harvested at 3cm from root.

Procedure

Nine to eleven days old wheat grass leaves was collected & cleaned with water and dried it in shaded area. 2 g of wheat grass leaves were cut into small pieces with scissors and put into the mortar.

3 ml of acetone was poured into the mortar and grinded the residue with the pestle again. Liquid part was poured into the screw-cap 25 ml conical flask through a filter funnel with a piece of cotton wool in it. 3 ml of acetone and 5 ml of hexane was poured into the mortar and grinded the residue again and then filter the extract into the screw-cap conical flask. The residue left over should be a very pale green. 3 ml of brine was added into the screw-cap conical flask. Cap was put tightly and the screw-capped conical flask was kept in microwave oven. Oven was set for 60 seconds at convention. Conical flask was removed from oven. Chlorophyll was vigorously washed in acetone-hexane solution. Then the flask was set back on the stand and allowed the mixture to separate. The top layer was dark green and the bottom layer was found to be clear. Anhydrous sodium sulphate was added to the solution containing the chlorophyll inside the screw-cap test tube to dry the solution [17] and subjected to spectroscopic studies.

Antibacterial and antifungal studies

Chlorophyll was dissolved in dimethylsulphoxide (DMSO) at a concentration of 1 mg/ml and used as working stocks. Ampicillin (25 µg) for bacteria, and Clotrimazole (30 µg) for fungi were used as reference agents. Susceptibility test was determined by disc diffusion method [18]. The nutrient agar plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 min, 0.1% inoculum suspension was swabbed uniformly, and the inoculum was allowed to dry for 5 min. Chlorophyll was loaded on 6 mm discs. The loaded discs were placed on the surface of medium and the extracts were allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 h for bacteria and 30 ˚C for 48 h for fungi with yeast peptone dextrose agar. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeters.

RESULT AND DISCUSSION

Extraction and purification

The yields from 100 g of wheatgrass were 18-20 mg of chlorophyll (obtained in triplicate).

Identification and purity

The recognition and purity of the chlorophyll a extract was determined by UV-Vis and IR spectrometry.

UV-Visible spectra

Spectrum of the chlorophyll extract demonstrated several absorption bands between 200 - 400 nm indicating a mixture of several pigments. Presence of carotene [19], xanthophylls and chlorophyll [20] was observed.

IR spectra

IR studies were done as per method of Goodwin [21]. Carbonyl region is of most significance as observed by ligand absorption which are independent of metal ion in the centre of tetrapyrrole ring. In the sample, C-H vibrations were observed at 2971, 2928 and 2875 cm^{-1} demonstrating presence of ketone and ketone oxygen coordination to magnesium, which was observed at 1701 cm^{-1} and 1643 cm^{-1}. Apart from this, C-C , C-N and C-O vibrations were also observed. C-N vibrations of the tetrapyrrole ring were also observed at 1351 cm^{-1}.

Antimicrobial studies

Haphazard use of antimicrobial agents has lead to multiple drug resistance. Table 1 shows the results of antimicrobial effect of chlorophyll on six microorganisms. chlorophyll showed no activity against *C. albicans* but showed prominent activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella oxytoca,* however no activity was seen against *Pseudomonas aurigenosa*. From the results (figure 1 and 2) it is none worthy to describe the difference in growth inhibition which is due to presence of chlorophyll.

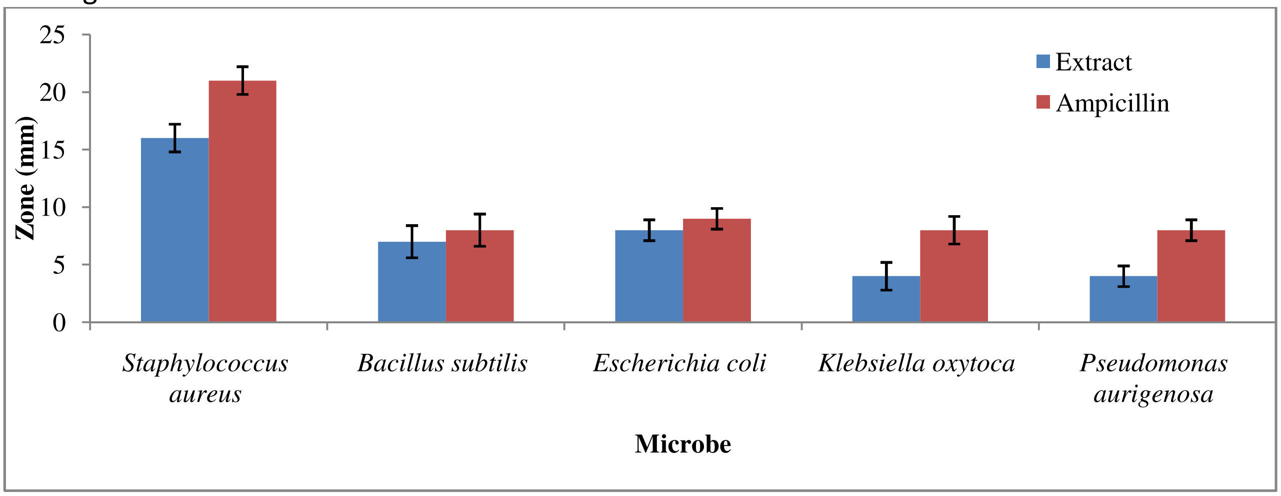

Figure1: Antibacterial activity of HEE chlorophyll on different microbes
Each value is expressed as mean±SD

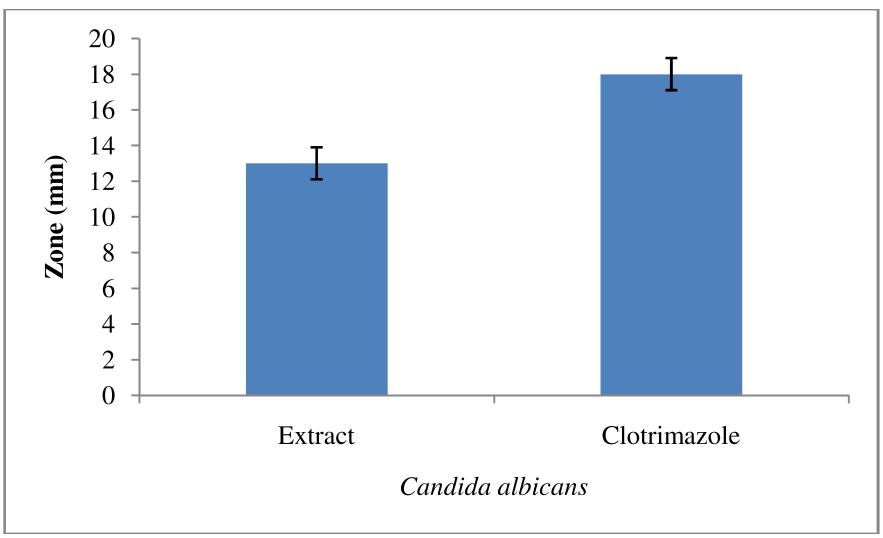

Figure 2: Antifungal activity of HEE chlorophyll on *Candida albicans*
Value is expressed as mean±SD.

CONCLUSION

The current work demonstrates successful isolation and purification of chlorophyll from wheatgrass. This is advantageous in terms of simplicity and time saving process. The study has also established antimicrobial potential of chlorophyll isolated from wheatgrass.

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