Extraction, Purification and Anti-Cancerous Activity of Lectin Protein from *Moringa oleifera*

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Abstract: ***Lectins belong to the class of proteins which specifically bind to certain carbohydrate moieties. The impact of lectins on human health and their potential benefits had been studied so far by several researchers till date. Here, in this work a thorough and simple method of extraction and purification of lectin protein from Moringa Oleifera has been developed. Also, the anti - cancerous activity of the protein has been checked due to its ability to target selectively cancer cells. This species is already popular in South - Asian countries for its high modulation to the immune system. This work suggests that the species proves to be one of the best gifts of nature.***

Keywords: Lectin protein, carbohydrate moieties, anti - cancerous and anti - bacterial, extraction and purification, Moringa Oleifera, blood lipid profiles, insulin secretion, polyphenols, bioactive properties, selective predation.

1. Introduction

The Moringa Oleifera is a fast - growing, drought - resistant deciduous tree belonging to the family Moringacae, native to the Indian Subcontinent. Its ability to withstand both severe drought and mild frost conditions enable its widespread cultivation in tropical and subtropical regions in many parts of the world. Another reason facilitating its extensive cultivation is its low invasive potential: Although listed as an invasive species by many countries, it has not been observed invading intact habitats or displacing native flora. Its cultivation usually requires a soil range of pH 6.3 to 7, and can be grown on altitudes ranging from 0 - 2000 meters. It is most suitable for dry regions, for it does not require expensive irrigation techniques and can be grown simply using rainwater. Moringa Oleifera is generally cultivated for its leaves, pods and kernels for oil extraction and water purification [1, 2].

Moving on, it is prudent to observe that the plant possesses notable nutritive and medicinal properties. Phytochemicals are present in high amounts in raw seed flour and amino acid content is at its peak in fermented and germinated seed flour. Moreover, boiling of Moringa extracts increases the availability of iron and anti - oxidant nutrients and reduces the concentration of cyanide, oxalate and phylate, thereby ensuring maximum utilization of required nutrients from the seeds and leaves. Owing to this nutritive property, the plant extract can be used to treat problems related to malnutrition. In addition, a recent study by Kiranawati on the effects on mammary glands of rats upon consumption of Moringa noodles indicated improved lactogogum values, thereby increasing milk production in the rats [3, 4]. This result is owed to the observation that the oil from the Moringa extract used in the experiment was rich in sterols [5]. The plant extract has also been incorporated in chocolates: 20% is considered an ideal incorporation in cocoa powder for the same. Furthermore, Moringa incorporation in halawa tahinia increased the nutrient value of the delicacy [6]. Indeed, the plant's nutritive properties have opened the doors for potentially developing protein and minerals - rich chocolate and halawa tahinia. Such Moringa fortifications may help ensure intake of adequate amounts of nutrients in children [7]. Owing to the realm of its medicinal properties, Moringa can be used to cure more than 300 diseases, and has long been used in herbal medicine by Indians and Africans. Indeed, the presence of phytochemicals makes it a good medical agent [8]. For instance, Moringa has been shown to cure both Type - 1 and Type - 2 diabetes. Specifically, the antioxidants in the plant helps reduce the ROS caused in Beta cells due to STZ induction during Type - 2 diabetes [9]. This can be understood through the fact that antioxidants like flavonoids and phenolics in Moringa tend to have a scavenging effect on the ROS, thereby keeping hyperglycemia under control and hence protecting the Beta cells. Moringa can also be used to prevent secondary ailments caused due to diabetes like retinopathy, nephropathy and atherosclerosis [10]. On another note, the plant can be used to treat cerebral ischemia due to its ability to act as a neuroprotectant. As a promoter of spatial memory, it can help in treating dementia. As an antiulcer agent, it can help in preventing gastric ulcers. In addition, a study by Viera proves that the presence of pterygospermin, moringine and benzyl isothiocyanate in the plant extracts can combat bacteria like Bacillus Subtilis, Staphylococcus Aureus and Vibrio Cholera [11].

Current research and experimentation aim to study the anti - cancerous activity of the Moringa Oleifera plant. It is believed that the wide range of phenolic compounds with great biological activity possess anti - cancerous properties that may be exploited to create medications for the treatment of selective malignancies. Quantitative RT - PCR analyses of Bax and BCL - 2 by DR. Kinjal Bhadresha, Dr. Vaidehi Thakore, Dr. Jpan Brahmbhatt, Dr. Vinal Upadhyay, Dr. Nayan Jain and Dr. Rakesh Rawal indicated abnormal expression of these genes under treatment conditions (Advances in cancer biology - Metastatis). Interestingly, the Moringa Oleifera leaf extracts may inhibit cell proliferation in the human cancer cell line A549 in a dose - dependent manner. Further morphological studies indicated that the extract stimulated apoptosis, as displayed by cell shrinkage, blebbing, chromatin condensation and nuclear fragmentation of cancer cells [12 - 14]. Research has examined how it might affect blood lipid profiles and insulin secretion. Extracts from leaves contain various polyphenols, which are

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under basic research to determine their potential effects in humans. Further considerable preliminary research has been undertaken to determine the presence of bioactive properties in the components of the Moringa Oleifera [15]. This manuscript builds on the work of previous research to examine how lectin extracts from Moringa Oleifera may foster anti - cancerous and anti - bacterial activity through selective predation of infected cells. We believe that if proven successful, this species may open doors for further breakthrough in research and harness potential solutions to fight selective cancerous diseases [16].

In this work the author has aimed to extract the protein by using a simple protocol initializing with salt precipitation followed by the ion - exchange chromatography. Principle of well - established chromatographic technique helped in eluting the protein of interest from column. The protein was used to predict its anti - cancerous activity as mentioned in various literature reviewed during this work. Human breast cancer cell of 43 - year - old white female patient with brest adenocarcinoma (AU565) plates were precured from ATCC [CRL - 2351] [17]. Inhibition halos were formed using different concentrations of protein. Standard Bradford essay was also performed to detect the protein concentration and found to be 2μg/μl.

2. Materials and Methods

2.1 Extraction

Dry Moringa seeds were purchased from an online shopping site.50 gm of seeds were grounded using a blender and dissolved in 200 ml of buffer (50mM Tris - HCl pH 7.5). After 20 minutes the solution was processed for centrifugation at 8000g for 15 minutes. The supernatant was further collected and stored at 4˚C.

2.2 Ammonium - sulphate Precipitation

The stored supernatant was treated with ammonium - sulphate. Firstly, 30% saturation was performed and kept overnight. Next day, the solution was spun down and supernatant was collected and further 70% saturation was performed and stored overnight. The solution was then centrifuged to collect the pellet and stored at - 20˚C the following day.

2.3 Cation - exchange chromatography (Purification)

The purification of the protein was performed using cation - exchange chromatography, wherein the column was prepared using sodium polystyrene sulfonate resin. The resin was settled and equilibrated with 50mM Tris - HCl pH 7.5. The pellet was resuspended in the same buffer and loaded on the column, where the flow through was collected and absorbance was observed. The column was then washed with the same buffer and elution was performed using different concentrations of NaCl (0.1M, 0.2M, 0.3M, 0.4M and 0.5M).

2.4 Anti - cancerous activity

Human breast cancer cells (AU565) were cultured in DMEM supplemented with 5% FBS (fetal bovine serum) and 0.5% penicillin - streptomycin and incubated overnight at 37˚Cin a CO_2 incubator.2×10^3 cells per well were seeded in a 96 - well plate and then treated with purified MO protein with different concentrations and further incubated for 72 hours. MTT (3 - [4, 5 - dimethylthiazol - 2 - yl] - 2, 5 diphenyl tetrazolium bromide) solution was then added to each well and the plate was again incubated for 4 hours. The formazan crystals formed after the reaction were dissolved in DMSO and the absorbance was measured at 570 nm. OriginPro 2018 software was used to represent the result on the basis of IC50 value.

3. Results and Discussions

3.1 Cation - exchange chromatography

Purification using cation - exchange chromatography was performed. Elution with NaCl gradient helped to recover the protein with best optical density at 0.4M salt concentration as 0.998. The sample observed its maximum peak at the 3rd fraction of 25 mL of the total elution buffer (0.4M) passed. Consistent decrease could be observed in the fractions of the eluted sample as shown in the chromatogram (figure1).

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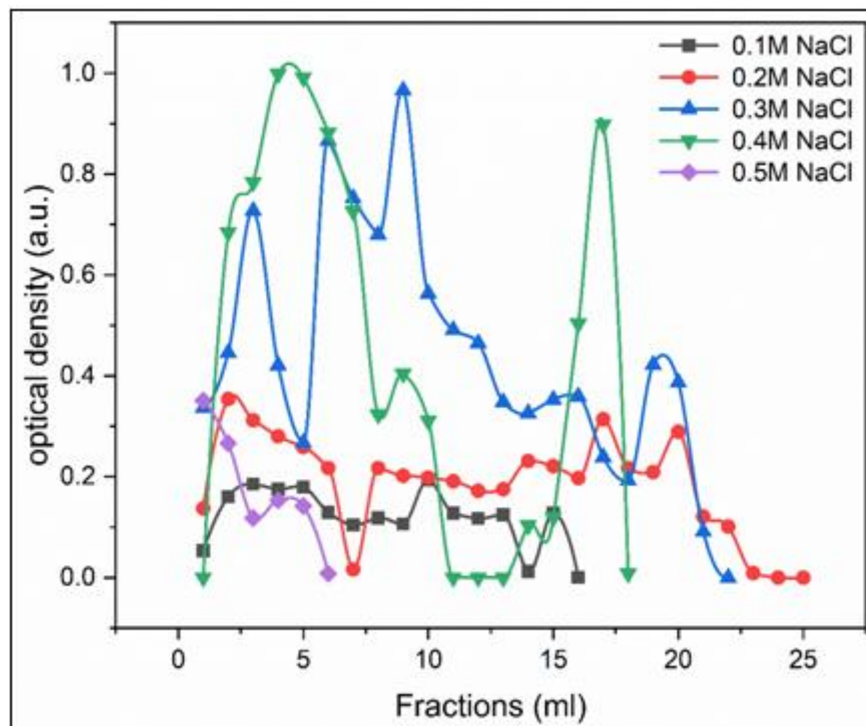

Figure 1: Chromatogram of purification using ion exchange chromatography

3.2 Purification

After purification the sample was concentrated using Amicon filter of MW cut - off 10 KDa. Little impurities were also found to be concentrated as shown in figure 2. The sample with highest absorbance that was found as 0.998 after chromatographic purification was ran on 12% gel. The bands were found at 32 KDa.

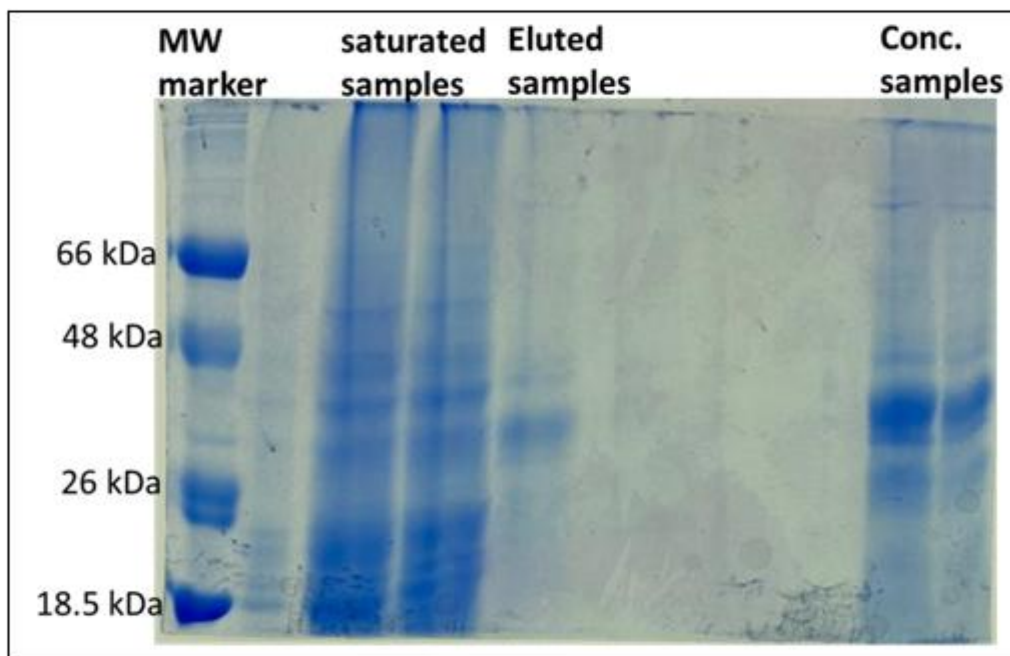

Figure 2: SDS - PAGE of marker along with purified protein at various stages

3.2.1 Salting - out (ammonium - sulphate precipitation)

Ammonium sulphate precipitation helped to precipitate out the pool of proteins.70% saturation salted - out the proteins by decreasing the pH of the reaction. Due to easy availability and solubility in water, proteins have been restricted to form bond with the water, precipitated out and collected in a pellet. The protein was quantified twice, once at this step after precipitation and second after obtaining protein of interest; the concentration was found to be 2µg/µl.

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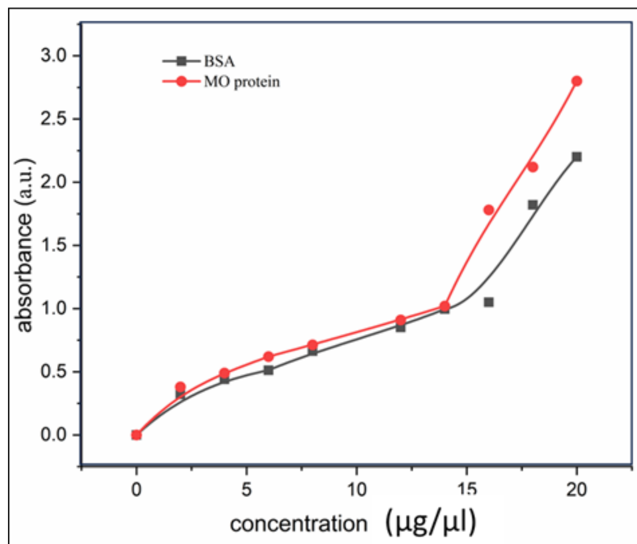

Figure 3: BSA based Bradford assay analysis for protein concentration quantification

3.2.2 Inhibition - activity

Effects of inhibition were studied through statistical studies. Maximum inhibition was depicted with 300µl of protein with 71% anti - cancerous activity. The anti - oxidants, high amounts of flavonoid, tocopherols and phenolic compounds attribute to the anti - proliferative properties of protein extracts on cancer cells. IC50 values were further observed using different concentrations of protein starting from 50 - 300 µg/ml. The Statistical representation of the significant cytotoxic effect are depicted in figure 4.

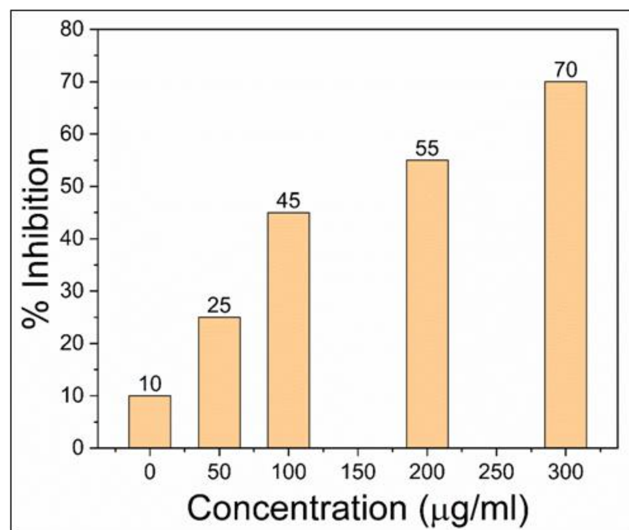

Figure 4: Percent inhibition effects on cancer cells

4. Conclusion

The MO protein was produced and purified to homogeneity using ion - exchange chromatography. Anti - cancerous activity has indicated that the protein has inhibitory behaviour towards the cancer cells. Furthermore, crystallization of this protein could be attempted to obtain the three - dimensional structure to design the drugs and further use in inhibition of the cancer proliferating cells. A striking application of this approach could be to combat Breast cancer: a matter of concern for decades. Hence, it is crucial to discover and design a drug which could be promising for the treatment of carcinoma cells. The MO protein unfurls optimism for presenting an evolution in the biological world of cell cancer. The evaluation of anti - proliferative efficacy of this protein indicates that it may be used as a therapeutic drug for selective cancerous diseases.

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