RESEARCH
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PREPARATION OF DEACETYLATED CHITIN FROM GLADIUS OF *Lolious investigatoris* (GOODRICH, 1896) AND EVALUATION OF ITS LEVEL OF REACTIVE OXYGEN SPECIES

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Abstract

Introduction: Chitin is the second most common substance found in nature after cellulose. It is mostly found in the cell walls of fungus, insects' cuticles, and crustacean exoskeletons. By removing an acetyl group from chitin using glucosamine and N-acetylglucosamine, chitin can be transformed into chitosan. Because of chitosan's strong biological compatibility, biodegradability, hemostatic activity, and wound healing function, it is widely employed in biomedicine. Antioxidants are chemicals that slow down or stop cellular substrates from oxidation. Finding natural antioxidants has attracted more attention recently because of their excellent properties and less side effects. To prepare deacetylated chitin from gladius of *Loliolus investigatoris* and evaluate its level of reactive oxygen species.

Materials and methods: Chitin was extracted from the pulverised gladius of *Lolious investigatoris* powder by demineralisation and deproteinization. Chitin thus obtained was converted into chitosan by the process of deacetylation. Post lyophilisation of the obtained precipitate, the deacetylated chitin was characterised through FTIR, SEM and XRD. The antioxidant properties of the prepared chitosan were assessed by employing 3 crucial assays, namely, DPPH radical scavenging assay, metal ion chelating assay and superoxide radical scavenging assay.

Results: The functional group properties of prepared chitosan were analysed through FT-IR spectroscopy and different bands were recorded. SEM analysis assessed the morphological parameters of the deacetylated chitin and revealed a smooth and non porous membranous surface. XRD analysed the crystalline structure of the extracted chitosan and various peaks at different intensities were analysed. The antioxidant activity of prepared chitosan was found to be around 65-75% of the different standards used in DPPH radical scavenging assay, metal ion chelating assay and superoxide radical scavenging assay.

Conclusion: The results of this study indicate that the deacetylated chitin derived from the gladius of *Loliolus investigatoris* has excellent structural and antioxidant properties and can be employed commercially after further confirmation through in vivo models.

Key words: *Loliolus investigatoris*, deacetylated chitosan, antioxidant property, gladius of squid, biological waste

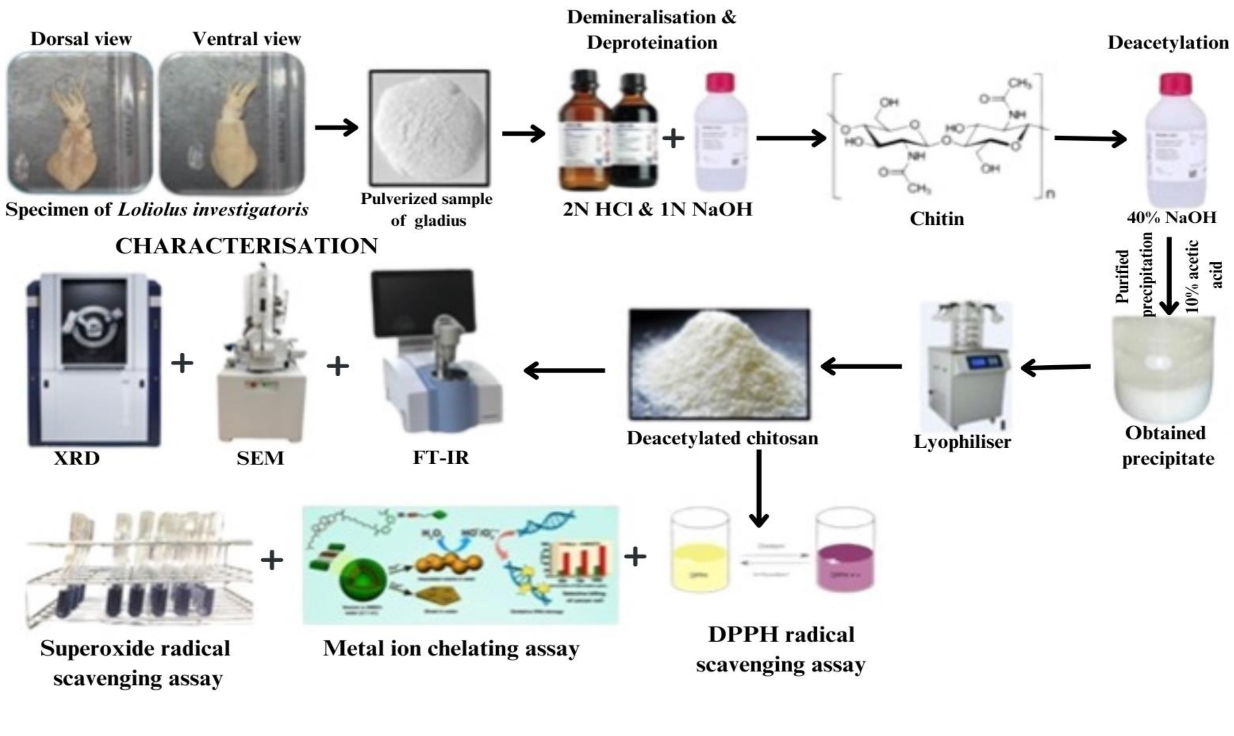
INTRODUCTION:

The health of the human body depends upon its ability to metabolise and oxidise lipids, proteins, carbohydrates and other biochemical molecules. The oxidation process of mitochondrial respiratory chain produces reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radicals, and superoxide anion as byproducts of normal cellular metabolism (Nathan, C. and Cunningham-Bussel, A., 2013). Normal levels of ROS have beneficial effects such as destruction of pathogens, wound healing, and repair (Bhattacharyya, A. et al, 2014). As a component of the cellular defence system, enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), thioredoxins (Trx) along with the non-enzymatic antioxidants are naturally produced against ROS (Rahman, I et al, 2006). At times, the cellular natural antioxidants fail to deal with the abnormal synthesis of ROS causing this oxidative stress to generate an array of pathologies such as ageing, diabetes, tumours, wrinkles or creases, autoimmune diseases such as rheumatism, neurodegeneration and infections (Leung, P.S. and Chan, Y.C., 2009; Maynard, S., et al, 2009; Abbas, M. and Monireh, M., 2008; Pillai, S., Oresajo, C. and Hayward, J., 2005; de Vries, H.E., et al, 2008; Hur, J., 2010). By halting the free-radical chain oxidation and donating hydrogen, antioxidants are able to stop the radical chain reactions of oxidation, creating stable free radicals that won't start or spread additional oxidation of lipids (Rupérez, P. et al, 2002). In order for antioxidants to effectively scavenge free radicals in the human body, it is crucial to use and develop them. However, several of them, including butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT), are thought to be carcinogens or hazardous substances. The study of strong natural substances with minimal toxicity as antioxidants has recently grown in importance.

Cephalopods make up about 30% of all trawl landings in Maharashtra. As exploitation and fishing grounds increase, new catches of cephalopsids are being reported from all over the coast. Cephalopsids (Loliolus investigatoris, Goodrich, 1886) are commonly caught in trawls. Chitin is a naturally occurring homo-polysaccharide that is found in large amounts in the cuticle of insects, the shells of crustaceans, certain fungi, and the cell walls of some bacteria. Chitin is a water-insoluble substance that possesses intra- and inter-molecular hydrogen bonds and resembles cellulose in terms of solubility. With a high percentage of acetylated residues, chitin is a copolymer of N-acetyl glucosamine and N-glucosamine residues joined by -1,4 glycosidic linkages. A deacetylated version of chitin called chitosan has repeated D-glucosamine units connected by (1-4) glycosidic bonds. Its amino groups make it soluble in an acidic aqueous solution. Chitosan is an excellent choice for a variety of uses, from waste management to food processing, textiles, cosmetics, medicine, biotechnology, and more. It is also very easy to work with because it is soluble and biodegradable. It is also biocompatible and non-toxic, so it won't cause any harm to your body. In the medical field, a great deal of emphasis has been placed on chitosan, such as designing absorbable sutures based on its hemostatic activity, drug carriers, antitumor agents, hemostats, and wound healing agents (Muzzarelli, R.A.A., 1994).

Antioxidant properties of natural substances have been demonstrated to be effective in safeguarding against oxidative harm and disease (Hayati, M.A., 2011). Chitosan is one such natural substance that has been demonstrated to possess antioxidant properties, and a variety of its derivatives have been chemically synthesized and evaluated for their antioxidant potential. This research aims to prepare and characterise deacetylated chitin from gladius of Loliolus investigatoris in order to evaluate its antioxidant activity.

MATERIALS AND METHODS:



Flow chart 1: Preparation and characterization of deacetylated chitin

The current study was conducted in Saveetha Dental College for a duration of 3 months.

RESULTS:

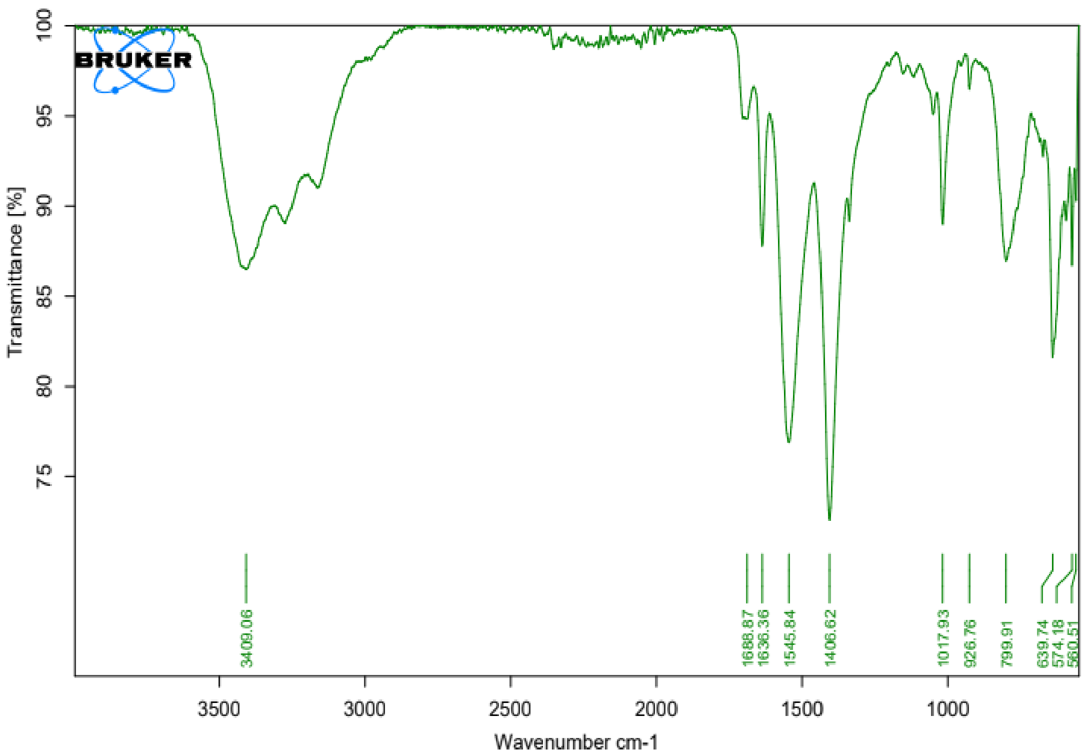


Fig: 1 FT-IR Analysis

The intensity of band at 3409.06cm−1 in the IR spectrum was due to the hydroxyl stretching vibration of the deacetylated chitosan and as expected they were broad. The weak absorption bands around 1545.84-1688.87 cm−1 were related to the C-H stretching vibrations of CH2 groups.

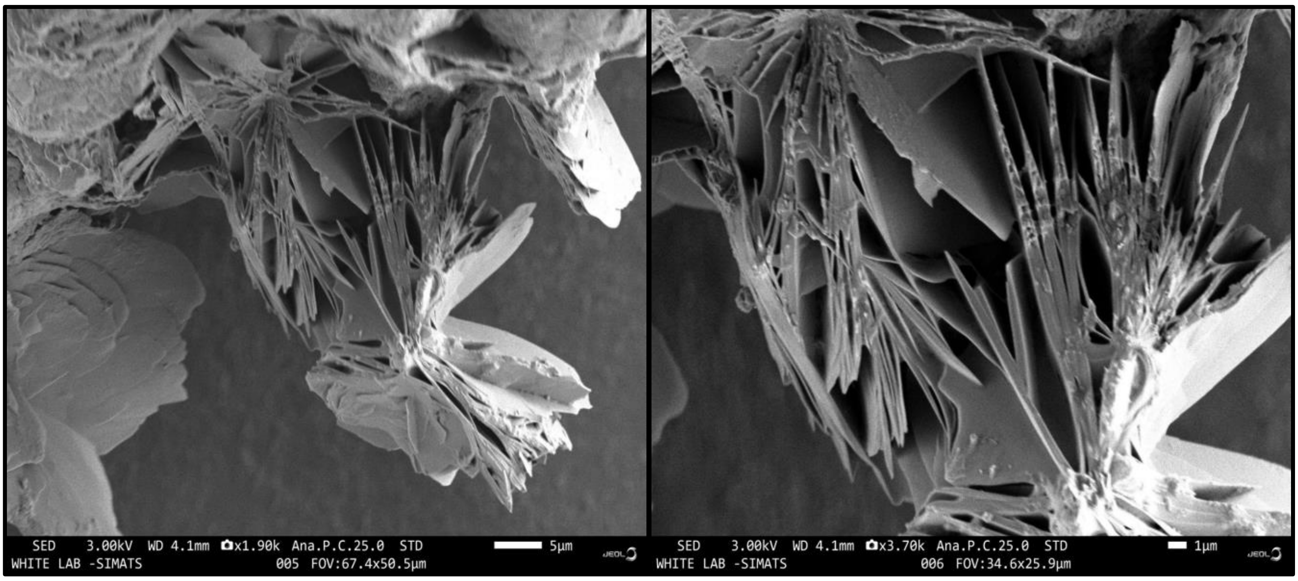


Fig: 2 SEM Analysis

The SEM images of deacetylated chitosan exhibited a nonporous, smooth membranous phase consisting of dome shaped orifices, microfibrils and crystallite. The electron micrographs of chitosan-chromone derivative gels exhibited a porous and chain-like shape.

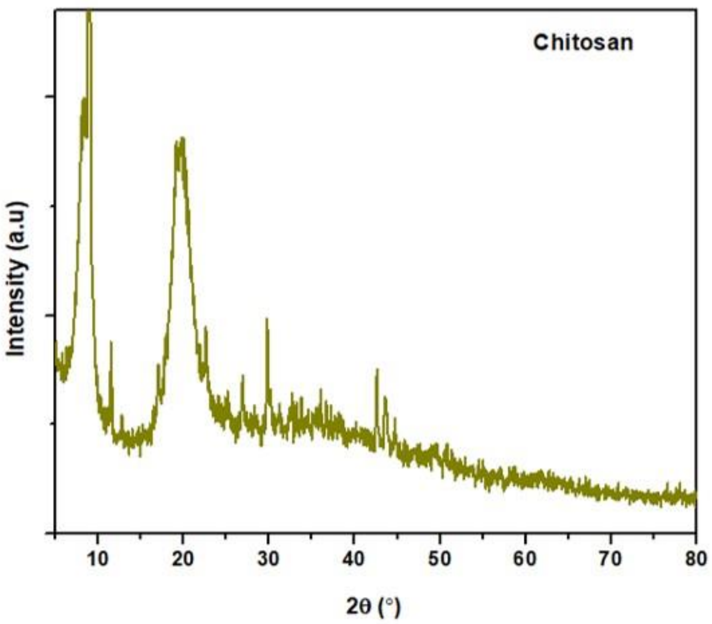


Fig: 3 XRD analysis

The deacetylated chitosan displayed two weak peaks at around 2θ of 20°and 35°. However, the peak observed for chitosan at 2θ = 10° disappeared and the very broad peak at 2θ = 20° became weak in deacetylated chitosan.

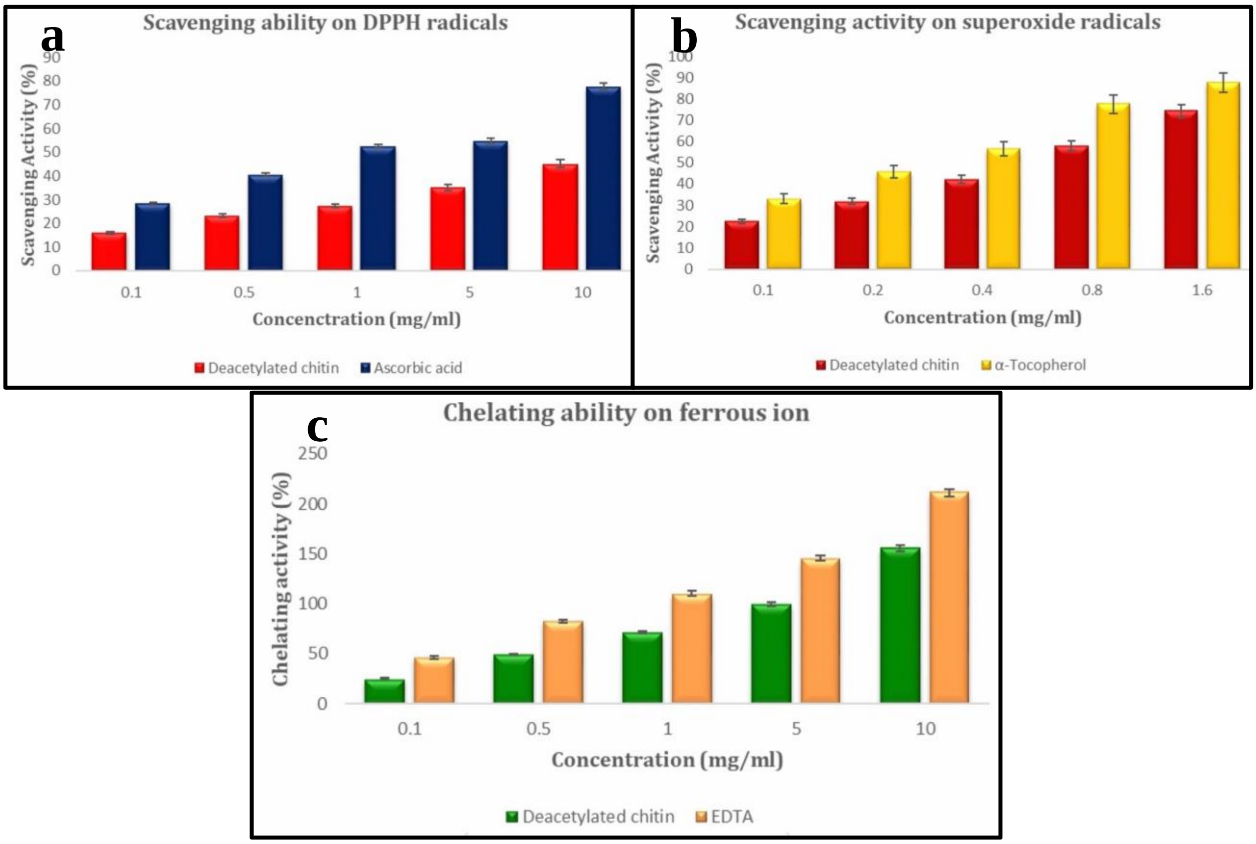


Fig:4 Antioxidant assays: (a) scavenging ability of DPPH randicals, (b) scavenging ability of superoxide radicals, (c) chelating ability on ferrous ion

DISCUSSION

Partially deacetylating chitin results in the formation of Chitosan, a non toxic and biocompatible cationic polysaccharide. Chemical modifications of Chitin and Chitosan for the purpose of creating new bio functional materials are of prime importance because such modifications would not alter the basic structure of Chitin & Chitosan while preserving the original physico-chemical and biochemical characteristics of the introduced group (Muzzarelli, R.A., 1973). There is very limited research conducted to assess the antioxidant properties of deacetylated chitosan. This study aims to prepare deacetylated chitin from the gladius of the squid, Loliolus investigatoris and to assess its structural properties along with its antioxidant activities.

Chitosan structural change after chemical treatment is confirmed by FT-IR with comparison to standard chitosan. Chitosan's Hydroxyl Stretching Vibration (HVT) is responsible for broad bands and intense bands in the infrared spectra (3409.06cm-1) The stretching vibrations of CHO and C = O bonds are attributed to the absorption in the following spectra, 1545.84-1688.87 cm^{-1} , respectively.

Surface Morphology and Microstructure of deacetylated chitosan was done using a scanning electron microscope (SEM). The SEM images of deacetylated chitosan exhibited a nonporous, smooth membranous phase consisting of dome shaped orifices, microfibrils and crystallite. The electron micrographs of chitosan-chromone derivative gels exhibited a porous and chain-like shape.

An XRD analysis was conducted to determine the crystalline structure of deacetylated Chitosan. The relevant diffract data are illustrated in Figure 3. The intensity of the X-rays was determined as a function of Diffraction Angle 2 and the orientations of the specific materials. This diffraction pattern was used to identify the crystalline phases of the specimen, assess its structural properties, and determine the size and orientations of the crystallites (small crystalline regions). However, the peak observed for deacetylation of Chitosan (2 = 10°) was lost, and the very widely observed peak (2 = 20°) was reduced to a weak peak (2 = 35°).

Three different antioxidant assays were conducted to evaluate the antioxidant property of chitosan. The scavenging ability of deacetylated chitosan on dpph radicals, scavenging activity on superoxide radicals and chelating ability on ferrous ions was 65-75% as efficient as the respective standards.

CONCLUSION

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