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FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY OF AGAR FROM RED SEAWEEDS OF KARACHI COAST

Iqra Rasheed[1], Asma Tabassum[1], Usmanghani Khan[2] and Aliya Rehman[1]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*Herbion Pakistan (Pvt.) Ltd., Karachi, Pakistan*

ABSTRACT

Agar was extracted from red seaweeds (*Gracilaria corticata* and *Gelidium pusillum*) of Karachi coast and its chemical bonding was analyzed by FTIR spectroscopy for the first time from this area. In a pre-extraction step seaweeds were treated with acetic acid, formalin and charcoal at two different time intervals 5 hrs and 12 hrs, to determine its effect on extraction of agar. Formalin treatment showed higher yield of agar as compared to others treatment.

Key words: Seaweeds, Rhodophycota, Agar, FTIR (Fourier transform infrared spectroscopy).

INTRODUCTION

According to Kelman *et al.* (2012) phycocolloidal substances are enclosed in cell wall and matrix of the seaweeds to provide them protection against wave current and harsh environmental conditions. Red seaweeds are economically important for production of agar and carrageenan (Gavino and Torono, 1999; Jantana *et al.*, 2006), as they are not present in any other land plant (Carté *et al.*, 1996).

Commercially agar has been well recognized worldwide (Kumar *et al.*, 2009) as gelling, and stabilizing agent for food, pharmaceutical, cosmetics and paper industry (Sabra and Deckwer, 2005; Brownlee *et al.*, 2012). Many well developed countries mainly Japan, Malaysia, China, Brazil and USA have been reported for harvesting and cultivation of seaweeds to fulfil the industrial demand (Edis, 2013; Fitton, 2008).

Pakistan has a coastline of about 1000 km bordering northern pole of Arabian Sea (Thompson and termizi, 1988), which shows luxuriant growth of seaweeds (Rizvi and Shameel, 2001). A lot of research has been carried out on the biochemistry and morphology of seaweeds from this region (Usmanghani and Shameel, 1996; Shaikh and Shameel, 1995; Ali *et al.*, 2000, Valeem and Shameel, 2007; Shahnaz and Shameel 2009). Shameel (1996) considered that seaweeds reported from Pakistan coast are potential source of biochemical compounds and phycocolloidal substances such as agar, carrageenan and alginate (Mahmood and Siddique, 2010; Khan and Qari, 2012; Javed *et al.*, 2015; Zahid *et al.*, 2017).

Although a lot of research has been carried out on taxonomy, distribution, bioactivity and composition of red seaweeds of Karachi coast (Rizvi and Shameel, 2005; Tariq *et al.*, 2011; Hanif *et al.*, 2016), but very few reports have been published on extraction of agar from Pakistan coast, Qari and Siddiqui (1993) investigated biochemical composition and yield of agar from *Gracilaria corticata*. Tabassum (2016) introduced the commercial aspects of seaweeds by utilizing agar from *Gelidium pusillum* for production of biofilms with enhanced quality.

Aim of this study was to determine the effects of different treatments on extraction of agar from *Gracilaria corticata* and *Gelidium pusillum*, Moreover a technique FT-IR spectroscopy was used for the first time for describing the chemical bonding of extracted agar from coastal area of Karachi.

MATERIALS AND METHODS

Collection of seaweeds

Seaweeds were collected during low tide from Bulleji coast Karachi during the year 2015-17. They were brought to the laboratory and rinsed with water several times until all the dust particles and puberties removed from the material. Seaweeds were blotted and allowed to dry at room temperature and stored in polythene bags.

Extraction of Agar

Agar was extracted by following the method of Hernandez *et al.* (2013) with some alterations. In a pre-extraction step, *Gracilaria corticata* and *Gelidium pusillum* samples were taken in triplicate manner and treated for 5 hours with acetic acid, charcoal, formalin and tap water as control and similarly performed for 12 hours treatment.

Treated seaweed material were washed with tap water then agar gel was extracted by traditional boiling in hot water. After the filtration of agar gel, extra water was removed by freeze-thaw method, gel was allowed to dry to obtain agar powder.

FTIR spectroscopy of agar

Two gm powder of extracted agar was mixed with potassium bromide to prepare a solid disc and FTIR spectra were collected from 4000-500 cm^{-1} range in transmission mode with 2 cm^{-1} resolution over 10 scans by using spectrometer (Shimadzu FT-IR-8900).

Statistical analysis

Results were statistically analyzed by two way ANOVA. Lowest significant difference (LSD) was evaluated at $p < 0.001$ for both yield of alginate and agar, Duncan's multiple range test was employed to compare treatment as mean value by using 'Statistica' software.

RESULTS AND DISCUSSION

Extraction of agar

Previous reports suggested that pre extraction treatment of seaweeds with alkali, formalin and acetic acid were proved to be effective for discoloration and better yield of phycocolloidal compounds (Istini *et al.*, 1994; Truus, 2001).

Earlier, phycocolloidal substances showed higher yield from pretreated seaweeds as compared to control (Jayasinghe *et al.,* 2016). However, in this study results recorded of both agarophytes *Gelidium pusillum* (Fig-1a) and *Gracilaria corticata* (Fig-1b) showed reduced pigmentation and significant increase in production of agar when treated with formalin and followed by acetic acid and charcoal.

It was also observed that extracted yield of agar was increased at 12 hrs treatment as compare to 5 hrs treatment. It is considered that, in pre-extraction step, time interval and temperature of treatments are also important factors, which have different effects on yield and gel strength of phycocollaidal substances (Arvizu-Higuera *et al.*, 2008). However results may vary from species to species and also yield of agar is influenced by environmental conditions and growing stage of seaweeds (Heydari *et al.,* 2014).

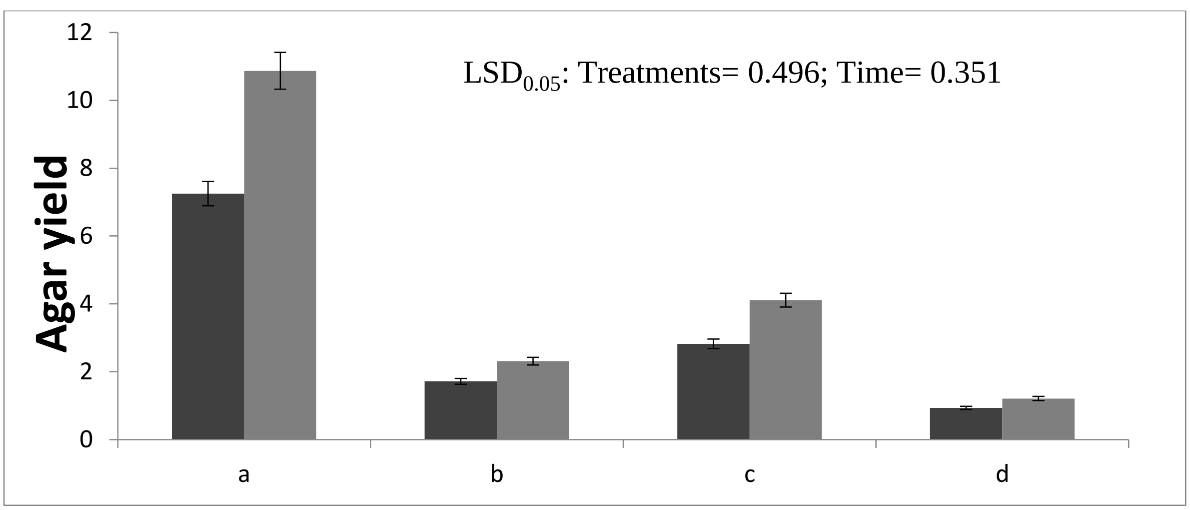

Fig 1a- Yield of agar from *Gracilaria corticata* under the effect of pre-extraction treatments.
Where, a= Formalin, b= Charcoal, c= Acetic acid, d= Control. = 5 hours = 12 hours

FT-IR spectroscopy of agar

This is the first report of its kind from this area of study, in which FT-IR spectra was used for indicating the chemical bonding of extracted agar. FT-IR spectra of agar showed vibrational peaks at 3419.3 cm^{-1} (Fig-2a), and 3421.9 cm^{-1} (Fig-2b) which indicates the presence of O-H stretching, furthermore absorption band was found at 2900 cm^{-1} is associated with methoxyl group, while vibrational band occurred at 1600 cm^{-1} presented the CO and NH groups, which are responsible for the formation of conjugated peptide bonds (Esam *et al*., 2012). It is also observed that the presence of band at 930 cm^{-1}, 1073.3 cm^{-1} and 1072.3 cm^{-1} (Fig-2a and 2b) indicates the presence of 3, 6-anhydrogalactose bridges, which confirms the composition of extracted agar, previous reports also showed similar results for FTIR of extracted agar (Pereira *et al*., 2013).

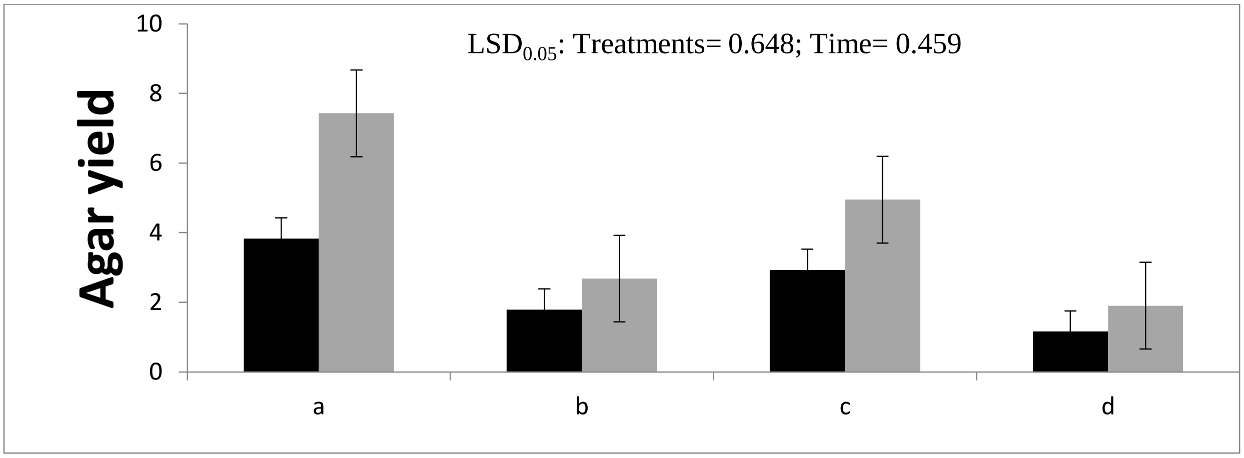

Fig 1b- Yield of agar from *Gelidium pusilum* under the effect of pre-extraction treatments.
Where, a= Formalin, b= Charcoal, c= Acetic acid, d= Control 5 hours 2 hours

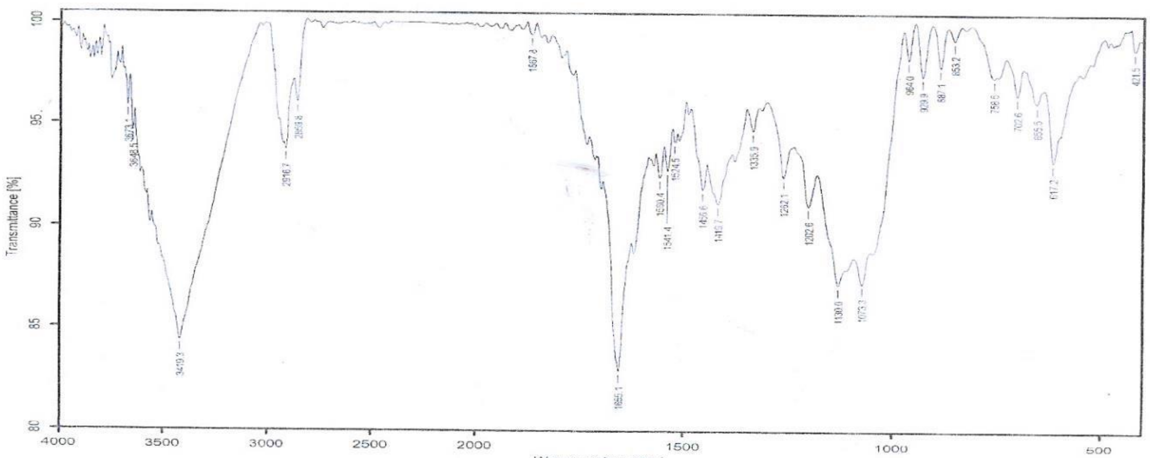

Fig 2a- FT-IR spectra of agar from *Gelidium pusillum.*

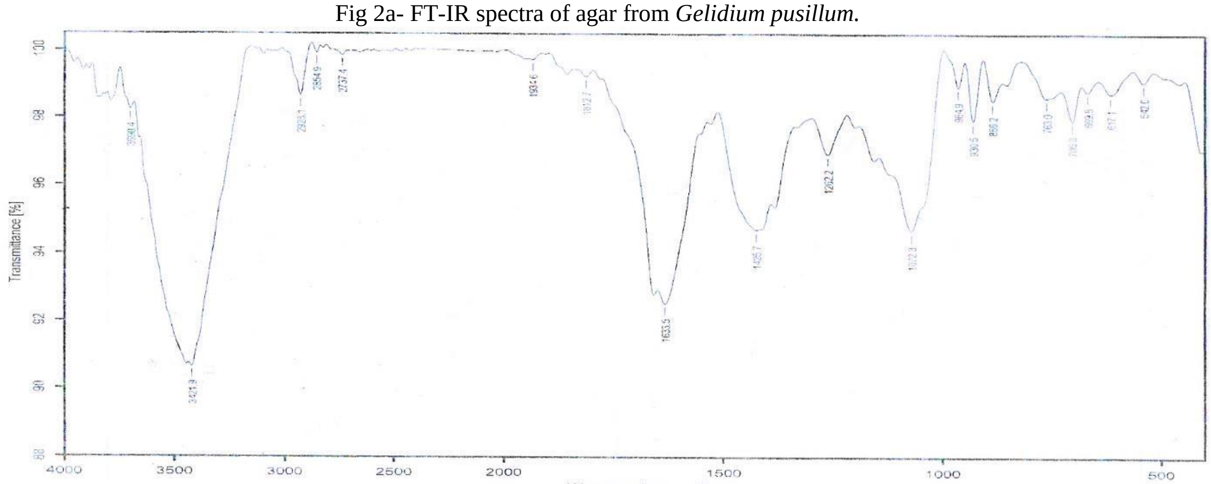

Fig 2b. FT-IR spectra of agar from *Gracilaria corticata.*

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