5.0. EXPERIMENT-1

Extraction of the pectic polysaccharide (CCPS) from *Momordica charantia* (Bitter gourd) and its characterization.

5.1. Objective of the investigation

1. Extraction and isolation of pectic polysaccharide (CCPS) from *Momordica charantia* (Bitter gourd, Karela).

2. To study the association of CCPS with sodium arsenite (As^{III}).

3. The present experiment also intended to explore the characterization of CCPS and its corresponding association with As^{III}.

5.2. Results

5.2.1. UV-Visible spectroscopic study

The UV spectrum of CCPS illustrated absence of peak (Fig 5.1a) whereas arsenic-CCPS combination confirmed two narrow as well as sharp peaks (Fig 5.1b).

5.2.2. FT-IR Spectroscopic study

The spectral characteristics of CCPS and arsenic-CCPS association were revealed in the infrared spectra (Figs. 1B.a-b). The above spectra transmitted the signals in the typical wave numbers range of 400-4000 cm^{-1}. The four main regions were documented and characterized to determine the above spectra. These key regions are:

(a) 3000–3600 cm^{-1} (b) 2800–3000 cm^{-1} and (c) 800–1500 cm^{-1}.

Here, the strong as well as the broad absorption bands are situated at 3575 to 3170 cm^{-1} of CCPS sample which due to the hydroxyl groups (O-H) (Chokboribal et al., 2015; Chawananorasest et al., 2016). Other two absorption bands of CCPS are presented around 2931 and 2368 cm^{-1} which are coming due to the existence of alkyl groups (C–H bond). It was earlier proved from the presence of sugar ring in CCPS (Romdhane et al., 2017; Santhiya et al., 2002). The carbon-hydrogen (C–H) bond exsist due to the presence of a methyl ester

group (OCH3) (Fig 2.a). The two spectral peaks position at 1760–1730 cm^{-1} and 1650–1600 cm^{-1} are generally allocated to the ester carbonyl (C-O) along with unesterified carboxylate (COO^{-}) ions group in a different order (Chatjigakis et al., 1998; Manrique and Lajolo, 2002). De Paula 1998 reported that the weak band position at 1736 cm^{-1} corresponds to the C=O stretching vibration mood of the carboxylic group (C=O) of galacturonate acid and the strong band position between at 1650–1600 cm^{-1} corresponds to the aromatics of C=O and C=C vibrations. Our results are similar in its pattern of band position and this is in agreement with the earlier findings (Zhang et al., 2018) (Fig 2.a). The spectral characters are noted in the FTIR spectrum pattern of the CCPS in the fingerprint regions (800-1500 cm^{-1}) which are mainly assigned to the vibrational position of the glucose monomer at the spectral position at 1300-1500 cm^{-1}. It corresponds to the vibrational modes related to the bending and deformations of the groups containing a carbon and hydrogen atom (Kizil et al., 2002; Deeyai et al., 2013). Our results showed that the vibrational band position at 1248 cm^{-1} which (Fig 5.2.a) is attributed to the C-O-H deformation approach. A different vibrational band position at 1160 cm^{-1} is assumed to be assigned to the coupling modes of C–C and C–O stretching mode. The α- and β-conformers of carbohydrates could be distinguished based on vibrational modes in the anomeric region from 750 to 1000 cm^{-1} (Azmi et al., 2012) where the absorption bands are situated around 891 and 844 cm^{-1}. This is well known for the α-conformer and β-conformer respectively (Cunha et al., 2017). The spectra of As^{III}-CCPS association were also dominated by a broad-band position in the same region for the stretching vibration modes of C-O and O-H groups (Fig 5.2.b). However, this interaction is assigned to the C-H which confirms no presence of protein (Zhang et al., 2018).

5.2.3. XRD spectroscopy

Multiple diffusing regions of spectra have also observed in CCPS-arsenic association (Fig 5.3b). When x-ray diffraction was passed through in CCPS which is compared with the free

CCPS (Fig 5.3a). Whereas, in free CCPS structure, was not attributed in any diffused regions of spectra. It is also to be noted that, diffraction regions are attributed in the presence of arsenic (Fig 5.3b).

5.2.4. ^{1}H NMR spectroscopy

Here the CCPS structure has been analyzed using the ^{1}H NMR spectrum. The signals in the regions of 5.50–4.90 and 4.90–4.30 ppm were devoted to the presence of α-anomers and β-anomers protons. These results have confirmed that α-glycosidic and β-glycosidic linkages have existed in CCPS. Signals in the range of 5.12-4.90 ppm in CCPS (Fig 5.4a and 5.4b) spectrum have been attributed to galacturonate acid residues (Peng et al., 2012).

5.2.5. SEM & TEM study

The SEM study has primarily highlighted the difference between the CCPS and arsenic-CCPS association. It could also be understood from Fig 5.5A and 5.5B. The difference between the CCPS and arsenic-CCPS has confirmed the diffraction image of the TEM. It can be seen in Fig 5.5D. The circle along with the white spot is identified only for the arsenic-CCPS. The corresponding arsenic-CCPS composition planes were also identified. The smaller circle stands for As (102) whereas the larger one correspondence to As (212). The black spots of the TEM image see fig. 5.4C is due to the presence of this metalloid present in CCPS (Fig 5.5C).

Figure 5.1.

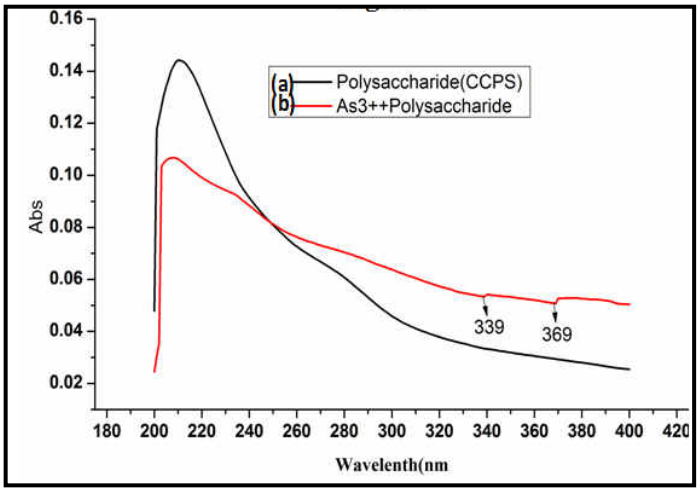
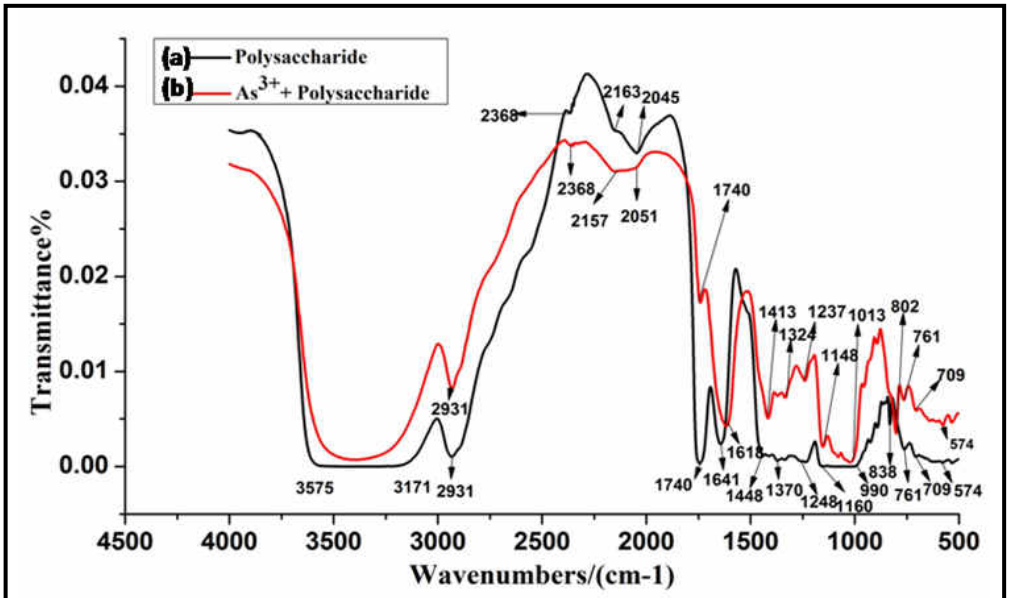


Fig 5.1. Represents the pattern of UV-visible spectroscopy absorbance of CCPS and As^{III} + CCPS association. (a) Indicates the UV-visible spectrogram absorbance of CCPS and (b) Indicates As^{III} + CCPS association.

Figure 5.2.



Difference in the peak position

CCPS	As^{III+} +CCPS
1641	1618
1448	1413
1370	1324
1248	1217
1160	1148

Fig 5.2. Represents the Fourier transforms infrared spectrum (FTIR) of CCPS and As^{III} + CCPS. (a) Indicates the FTIR spectrum of CCPS and (b) Indicates the FTIR spectrum of As^{III} + CCPS association.

Figure 5.3.

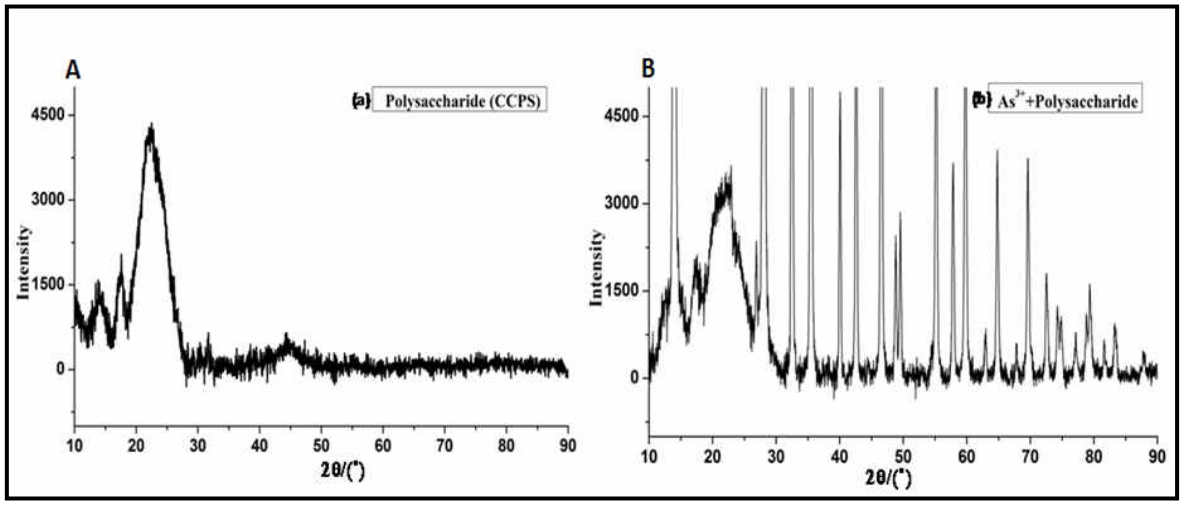


Fig 5.3. Represents the X-ray diffraction spectroscopy patterns of CCPS and As^{III} + CCPS association. (a) Indicates the XRD pattern of CCPS, and (b) Indicates the XRD pattern of As^{III} + CCPS.

Figure 5.4.

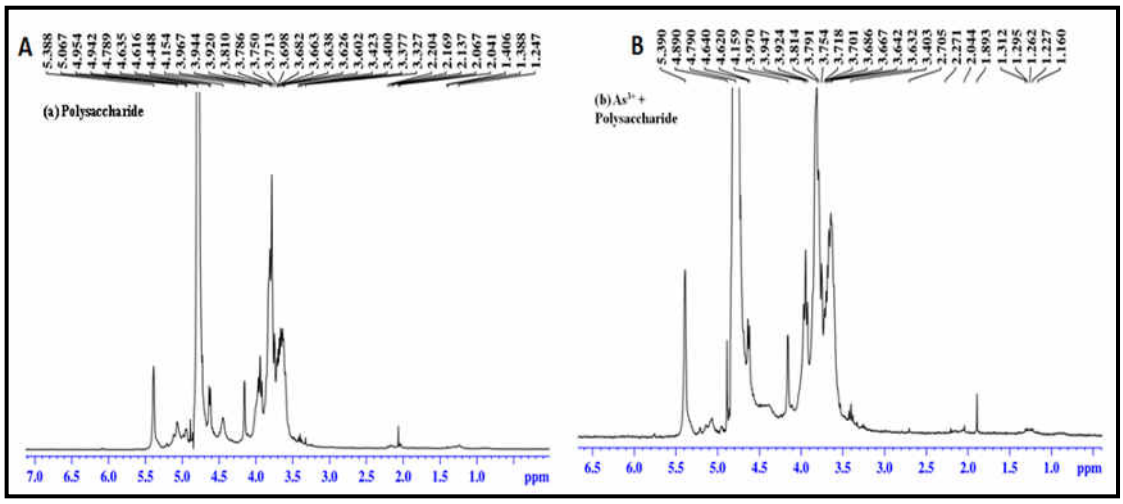


Fig 5.4. Represents the NMR spectrum patterns of CCPS and As^{III} + CCPS association (a) Indicates the ^{1}H NMR pattern of CCPS and (b) Indicates ^{1}H NMR pattern of As^{III} + CCPS association.

Figure 5.5.

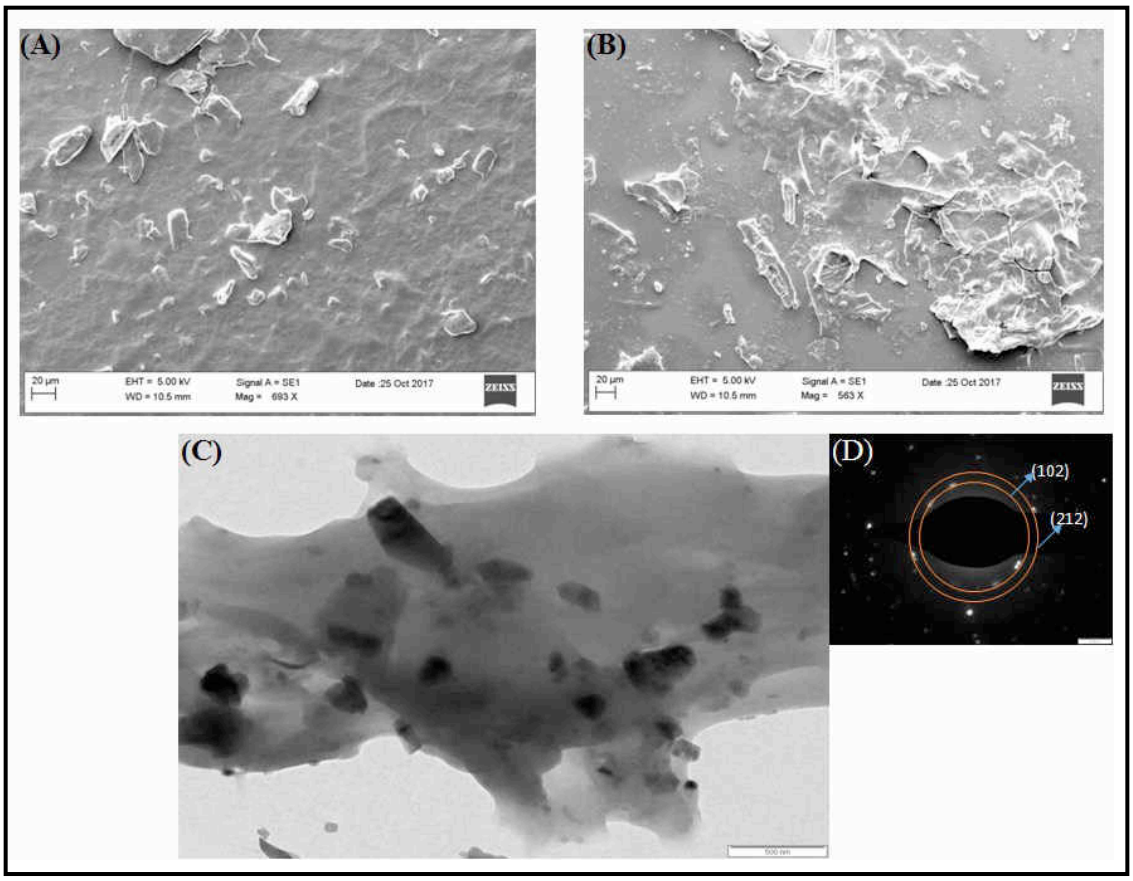


Fig 5.5. Represent the electron microscopic study of CCPS and As^{III} + CCPS association. (A) Indicates the scanning electron microscopy (SEM) of CCPS, (B) Indicates SEM of As^{III} + CCPS association, (C) Indicates the transmission electron microscopy (TEM) of As^{III} + CCPS association and (D) Indicates the Diffraction image of As^{III} + CCPS association.

5.3. Discussion

The mechanism of zinc chelation by this polysaccharide was reported earlier (Li et al., 2010). In this study we tested the binding capacity of polysaccharide from *Momordica charantia* with sodium arsenite *in vitro*. We observed the intensity changes and structural characterization of CCPS and arsenic-CCPS association by UV, FTIR, XRD and TEM analysis. Here we noted generation of the two narrow but sharp peaks of UV-spectrum due to the association of arsenic and CCPS but no such peak was found in case of pectic polysaccharide alone. These spectra have been elaborated in Fig 5.1.a and 5.1.b. Zhang et al

2018 reported that absence of the peaks at 180 between 260 nm reveals absence of protein. This information has confirmed the purity of polysaccharide. Similar type of result of the present investigation was also reported by Panda et al., 2015 where pure form of CCPS was obtained. The FTIR spectrum of CCPS and arsenic-CCPS association has been characterized based on the functional groups and different band peaks position i.e. peak position was upshifted and downshifted. These spectra have been elaborated in Fig 5. 2.a and 5.2.b. In the FTIR spectrum of CCPS with and or without arsenic association. Here, the spectrogram alteration including the band position shifting and the band intensity alteration are important factors for the characterization between the CCPS and arsenic-CCPS association. The comparison between the peak shift positions, i.e., the peak shift in the absorption bands of the bared molecule of CCPS and its arsenic bound complex indicates the possible interactions between the functional groups. The position of infrared peaks of polysaccharide alone was observed at 1641, 1448, 1370, 1248 and 1160 cm^{-1} (Fig 5.2.a). The sodium arsenite-CCPS associated peak positions have been also observed at 1618, 1413, 1324, 1237 and 1148 cm^{-1} region respectively (Fig 5.2.a.). All the above mentioned bands illustrate the downshift of 11-46 cm^{-1} in case of CCPS-sodium arsenite association in comparison with pure form of CCPS. These huge down and up shifts in the peak positions indicated the involvement of the functional groups like carboxyl, carbonyl, and hydroxyl groups during CCPS and sodium arsenite association (Fig 5.2.b). The changes in the band intensity have been also observed along with the shift in the corresponding band situation. Shift in the infrared bands accompanied by an alteration in intensity associated with these functional groups. It advocates the direct interaction of sodium arsenite with the moieties of these functional groups of pure CCPS. The functional group of this spectrogram alteration and the band intensity changes specify that CCPS bears possible chelated property due to the presence of potential reactive sites in its structure. In case of CCPS, the broad absorption of peak position

is attributed at 3575 to 3170 cm^{-1} indicates the presence of a hydroxyl group. Cheng et al 2013 reported the free radical scavenging activity of Momordica polysaccharide as a consequence of the functional group (hydroxyl) in its structure that acts as hydrogen donor. Finally, this functional group maintains oxidative related effects (Li, et al., 2010). Zhao et al 2013 reported that the hydroxyl group (O-H) polysaccharide may chelate the metal ions. Presence of a negatively charged galacturonate acid residues in CCPS enables to contribute an excellent potential action for the cation chelation.

In XRD analysis the diffusing region's absence or changes are essential for the differentiation between the elements including CCPS and arsenic-CCPS association. The absence of the diffuse regions for CCPS has also obtained in the XRD analysis which further indicates the purity of this CCPS. Possible chelation of arsenic with CCPS might be confirmed from this diffused nature of the qualitative spectrum of XRD (Fig 5.3a). The qualitative spectrum of the sodium arsenite-CCPS (Fig 5.3b) indicates the presence of arsenic and it was widely presented in the chelated sample. Moreover, ^{1}H NMR spectrum of the CCPS has confirmed the existence of α-anomers and β-anomers/α-glycosidic and β-glycosidic linkages with substantive glucuronic acid residues (Peng et al., 2012).

Hence we conclude that galacturonate acid residue and hydroxyl group in CCPS may contribute a potential interaction with sodium arsenite. These findings may help to develop a therapeutic strategy to combat against the arsenic mediated health hazards.