## *Original Research Article*

# *Holothuriaatra*:AnUnderutilizedMarineResource forNutritionalandCollagenBenefits


**ABSTRACT**

**Aims:**Toassessthenutritionalcontentinthebodywallofanunderutilizedseacucumberspecies:*Holothuri aatra*andtoextractandcharacterizethepotentialcollagentypesfromthebodywall**Studydesign:**Thespecimensofseacucumberwereidentifiedbasedonmorphologyandthenutritionallyanalyzedwithrespecttolipidprofile,vitamins,minerals,andcarbohydrates.*H.atra*bodywallwasusedtoextractcollagenandlatercharacterized.
**PlaceandDurationofStudy:**SampleswerecollectedatMannar,SriLanka.Studywasconducted at Department of Zoology, University of Sri Jayewardenepura, Sri Lanka during 1 year.**Methodology:**Nutritionalcontentinthebodywall(proximateanalysis,lipidprofile,vitamins,minerals,andcarbohydrates)wasanalyzedusingbiochemicalassays.Moreover,*H.atra*bodywallwasusedtoextractcollagen,byacidsolublecollagenextractionandcharacterizedbyphysico-chemicalmethods.
**Results:**
Theresultsshowedthatbodywalltissuesof*H.atra*containedhighmoisturelevel(83.2%),proteins (10.2%) and low levels of fat (2.0%) and carbohydrates (2.1%). Further, fleshcontains,0.4%saturatedfattyacids,0.4%unsaturatedfattyacids,considerableamountofCalcium, Magnesium and Sodium. Collagen yield from the body wall was recorded as 0.95% andfibrilsobservedasirregularanddensewithalooseandporousstructure.AttenuateTotalReflectance-Fourier Transform Infrared Spectroscopy and Ultra Violet Vis spectroscopy indicatedthecollagenwasTypeI.
**Conclusion:** Resultssuggestthatunderutilized,non-commercial*Holothuriaatra*isapotentialnutritional source and it contains type I collagen. Further studies are underway to increase thecollagen yield from *H. atra*and to develop a collagen membrane which can be used in futureindustrialapplications.


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3 Keywords:*Holothuriaatra,*marine collagen,marine geneticresources

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## 2 1.INTRODUCTION

3 *Holothuriaatra*(Lollyfish)isanon-commercialseacucumberspeciesavailableinSriLankancoast,

4 especiallyalongtheNorth-Westregion[1].It has long beenconsumed in many regionsof the world

5 asapotentialbiologicalsourceagainstaging,oxidation,inflammation,microbialactivity,woundsetc

6 [2].*H.atra*fleshishigh in proteinand further,it containssignificant levelsof essential andnon-

7 essentialaminoacids[3].Sea cucumbers are knownto offer remarkablenutritional profile and have



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8 atractedtheattentioninbothculinaryandhealthperspective. Therefore,itisworthwhiletoinvestigate
9 fullnutritionalprofileofnon-commercial,underutilizedseacucumberspecies,suchas*H.atra*.Recent
10 research relatedtoisolating bioactivecompoundssuchas collagen,flavonoids,phenolic components,
11 terpenoids,saponins,alkaloids,acidmucopolysaccharideandtriterpeneglycosidefrom*H.atra*has
12 shownsufficientevidence forunique industrialvalues ofthisspecies [2].

13 Beinga promisingoptionamong“bluematerials”whichcansubstitutemammal-derivedcollagen,
14 marine collagenhasmanyapplicationsinthefieldsofmedicine, cosmetics,pharmaceuticalsandfood
15 industries[4].Further,theyarerichinantioxidantproperties,environmentallyfriendlyextraction
16 procedures,lowmolecularweight,minorregulatoryandqualitycontrolproblems,anegligiblenumber
17 of biological contaminants and toxins, low inflammatory response and excellent metabolic
18 compatibility,freeofinfectiousdiseases,freeofallergiesandavoidingreligiousconcerns[4].Dueto
19 itsuniquestructuralproperties,marinecollagenisbeingusedinfoodindustryasadditives,packaging
20 material,dietarysupplement,functionalfood,confectioneryanddessertswhileusedasa
21 multifunctionalbiomaterialintissueregeneration,wounddressing,etc[5].Bioactive propertiessuch
22 asanti-agingandanti-wrinklingactivitiesenabletheuseofcollagentoformulatelotionsandgelswith
23 highmoisturizing action with UV protective properties that areimportant in cosmetic industry [5].

24 Seacucumber-derivedcollagenfibrilsfrequentlyshowsymmetricalspindleshapeandshortlength[6].
25 Theyarebipolarmoleculeswithasurfaceassociatedwithproteoglycans[6].Covalentinternal
26 crosslinksareverysimilartomammaliancollagensyetpermanentcrosslinksareabsentinthe
27 structurewhichfacilitatesisolationofcollagen,avoidingmechanicaldamage.Moreover,absenceof
28 permanentcrosslinkshelptoslidepassoneanotherinshorteningandlengtheningprocessamong
29 fibrils[6].TypeI collagenisthe mostcommonand abundantcollagentypein marineinvertebrates

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withtwoequivalentα1andoneα2polypeptidechainswhichcomposedof1.1×300nmsizecollagen molecules[7].

Fullnutritionalprofileofseacucumberscanbeinvestigatedusingwellestablishedprotocols. Collagenwhichisanpotentialproteinofseacucumberscanbeextractedusingconventionaland novelmethods. Characterization of extracted collagenis essential prior to investigatingon commercial applications.However,presentworkaimedtoanalysethefullnutritionalprofileof*H.atra,* underutilized sea cucumber species in Sri Lanka and obtain collagen to examine their physicochemicalcharacteristicsandstructure.Investigationsoncollagensextractedfromthesea cucumberarescanty,comparedtoothermarinespecies.Thereisgrowinginterestonthenon-commercialmarinespecieswithrespecttobioprospecting.Accordingly,thepresentstudyprovides thebasisfornutritional quality,extractionandcharacterizationofcollagensfrom*Holothuriaatra*.

## 2.MATERIALSANDMETHODS

### 2.1Samplecollection

Livespecimenof*H.atra*wereobtainedcommerciallyfromMannar,SriLankaandstoredinice.The visceraofseacucumbersampleswereimmediatelyremovedandthebodywallwasdicedandkept at−20$^0$Cuntilfurtheruse.

### 2.2Nutritionalcompositionanalysisof*Holothuriaatra*

#### *2.2.1* <u>Proximatecompositionof*Holothuriaatra*</u>

Thedicedtissuesamplesof*H.atra*wereusedtotestproximatecomposition.The moisture,ash, proteinandcrudefibercontentweredeterminedaccordingtothePearson'scompositionandanalysis offoods[8].

*2.2.1.1Moisturecontent:*

Accurately,1gofsampleswereincubatedinpre-heatedovenat105°C.theweightwasmeasured after 3 hour of drying and the weight difference was calculated to obtain the moisture content.

*2.2.1.2Ashcontent:*

Accurately,1gofsamplewasincineratedinamufflefurnaceat550-600˚Cfor24hours.Theresulted ashwas weighed and the percentage was calculated.

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1 *2.2.1.3 Totalproteincontent*

2 TheKjeldahlmethodwasperformedaccordingtomethod981.10oftheAOAC[9].Approximately
3 50mgofrawmaterialwashydrolysedwith15mLconcentratedsulfuricacid($H_2SO_4$)containingtwo
4 coppercatalysttabletsinaheatblock(UDK149AutomaticKjeldahlNitrogenProteinAnalyzer)at420
5 ◦Cfor2h.Aftercooling,distilledwaterwasaddedtothehydrolysatesbeforeneutralizationand
6 titration.Theamountoftotalnitrogenintherawmaterialsweremultipliedwiththetraditional
7 conversion factorof 6.25.

8 $$Nitrogen(\%) = \frac{(standardacidvolume\text{-}mlblank) x N ofacid x 1.4007}{weightofsample(g)}$$

9 CrudeProtein(%)=TotalN(%)xConversionfactor(6.25)

10 *2.2.1.4 Totalfatcontent:*

11 Totalfatcontentwas determinedusingthe Werner-Schmidmethod byHill [10].Briefly,10gportionof
12 thesampleunderwentdigestionwithHCltofacilitatethereleaseoffat.Thefatwassubsequently
13 extractedusingpetroleumether.Followingtheevaporationoftheether,theremainingresiduewas
14 weighedand calculatethe fatcontent byusing followingequation.

1 $$\%Fatinsample = \frac{Weightoffatinsample}{Weightofsampletaken(g)} x 100$$

16 *2.2.1.5 Carbohydratecontent*

17 Carbohydratecontentwas calculatedby thedifference ofallother componentsmeasured [8].

18 %TotalCarbohydrate=[100−%(Protein +Fat+Moisture+Ash+Fiber)]

19 ***2.2.2 Lipidcompositionanalysisof*** ***Holothuriaatra***

20 Determinationofsaturated,monosaturated,andpolyunsaturatedfattyacidswithEicosapentaenoic
21 acid(EPA),andDocosahexaenoicacid(DHA),contentoftheseacucumberfleshwasanalysedwitha
22 Gaschromatograph [11](GCMS-TQGCHeadspace/AutosamplerTrace1300Thermoscientific,
23 Industrialtechnologyinstitute,SriLanka).Cholesterolcontentwasmeasuredbyfollowingthemethod
24 ofAssociationofOfficialAnalyticalChemists(AOAC)994.10[12]withaGaschromatograph(GCMS-
25 TQGCHeadspace/AutosamplerTrace1300Thermoscientific,Industrialtechnologyinstitute,Sri
26 Lanka).

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#### 2.2.3 Vitaminsanalysisof*Holothuriaatra*

Theconcentration of vitamin A was determined using analytical high-performance liquid chromatography(HPLC)(Methanol:acetonitrile50:50phase)followingAOAC2001.13[13] method andVitaminCwasdetectedfollowingthemethodAOAC2012.22[14]at the Industrial technology Institute(ITI), Colombo,Sri Lanka.

#### 2.2.4 MineralCompositionanalysisof*Holothuriaatra*

Minerals;calcium(Ca),magnesium(Mg),potassium(K),sodium(Na),zinc(Zn),selenium(Se), copper(Cu),iron(Fe),andmanganese(Mn)wereanalysedbyInductivelyCoupledPlasmaMass Spectroscopy(ICP-MS).Briefly,0.5gofsamplesweredigestedwithconc.$HNO_3$(analagrade)at200 $^{0}$Cfor20minutesfollowedbyfilteringanddetectionbyICP-MassSpectrometer(Agilent7900ICP-MS,Residualanalysislaboratory,Industrialtechnologyinstitute,SriLanka).

### 2.3Extractionofcollagenfrom*Holothuriaatra*bodywall

Collagenextractionwascarriedoutaccordingtothemethoddescribedby YuniatiandSulardiano[15] withslightmodifications.Briefly,coarselygroundseacucumbersamples(PanasonicAC300,Japan) incubatedindistilledwater(1:10w/v)for30minuteswithstirring.Inthepre-treatmentprocess, samplesweremixedandstirredwith50%alcohol(1:2w/v)for30minutes,washedwithdistilled wateruntilthepHwasneutral.SampleswereimmersedinHCl0.1Mand4mMEDTA(1:10w/v)for 24hoursstirringcontinuouslytomaintainstablepHandreduceminerals.Followedbywashingwith distilledwater,toeliminatenon-collagenousproteins,sampleswereaddedto0.1MNaOHsolution (1:10w/v)andkeptfor2days,changingtheNaOHsolutioninevery24hours.Sampleswere thoroughlyrinsedwithcolddistilledwateruntiltherinsedwaterbecomeneutral(pH7.0).Remaining pelletsweresubjectedtoisolationofcollagenbyincubatingin0.5Maceticacid(1:10w/v)for48 hourswithcontinuousstring.Supernatantwasseparatedbycentrifugation(HERMLEZ306)at6000 rpmfor20minutes.Thepelletswerere-extractedwith0.5Maceticacidfor24hours,both supernatantswerepooled,laterNaClwasaddedtosaltoutcollagenuntiltheNaClfinalconcentration of thesupernatantreaches1M.Precipitatedcollagen wasseparatedbycentrifugationat2000gfor 15 minutesandfreeze-dried(ilShinBioBaseFDS8512freezedryer).

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1 **2.3.1 CalculationofyieldofAcidSolubleCollagen(ASC)**

2 TheyieldofextractedASCwascalculatedbasedonthedryweightofstartingmaterialasper

3 followingequation,

4 $$Yield(\%) = \frac{Weightoflyophilizedcollagen(g)}{Weightof initialdrysample(g)} x100$$

5 ## 2.4Physico-chemicalcharacterizationofcollagen

6 **2.4.1 Determinationofmoisturecontent**

7 ThemoisturecontentwasdeterminedasperthemethodologybytheAssociationofOfficialAnalytical

8 Chemists[16]bydrying1g ofASC at105°C untila constantweight wasobtained.The moisture

9 contentwascalculatedbasedontheweightdifference.

10 **2.4.2 DeterminationofpH**

11 Approximately1gofcollagenwasdissolvedin70mlofdoubledistilledwaterandthepHwas

12 measuredusing a pH meter (Consort C6010 Multi-parameter analyser) [15].

13 **2.4.3 AttenuateTotalReflectance-FourierTransformInfraredSpectroscopy(ATR-FTIR)**

14 ASCsamplesweresubjectedtoATR-FTIRspectroscopyusingFTIRspectrometer(BrukerAlphaFT-

15 IRspectrometer,DepartmentofMaterialScience,UniversityofMoratuwa).Thespectraintherange

16 of600-4000cm$^{-1}$withautomaticsignalgainwerecollectedin16scansataresolutionof4cm$^{-1}$and

17 wererationedagainstabackgroundspectrumrecordedfromthecleanemptycellat25°C.Analysis

18 of spectraldata wascarriedout usingOMNICSpecta™datacollection softwareprogram.

19 **2.4.4 UltraViolet(UV)–Visabsorptionspectraanalysis**

20 Collagensolution(5mg/mL)waspreparedbydissolvingcollagenin0.5Maceticacid.Baselinewas

21 setwith0.5Maceticacid.UV-VisabsorptionspectraofASCsampleswererecordedusingUV-Vis

22 spectrophotometer(ModelThermoScientificGENESYS10SSeriesUV-Visspectrophotometer,

23 Instrument Centre, USJ)in the rangeof 190-600 nm.

24 **2.4.5 VisualizationofcollagenunderScanningElectronMicroscopy(SEM)**

25 TheinternalfibrearchitectureofextractedcollagenwasobservedunderSEM(CARLZEISSEVO18

26 ScanningElectronMicroscope,DepartmentofMaterialSciences,UniversityofMoratuwa).

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# 3.RESULTSANDDISCUSSION

## 3.2Nutritionalcompositionof*Holothuriaatra*

### 3.2.1 Proximateanalysis

Accordingtothisstudy,proximateanalysiswascarriedoutforfreshseacucumbersamplescollected fromMannar,SriLankawhichrecordedforthefirsttime.Themoistureamountoffoodcomponent whichisaknownparameterisanindexofitswateractivity[17].Fresh bodywalls ofsea cucumber*H. atra*resultedhighermoistureas83.2%andfoundtobeinaccordancewiththepreviousworkwith severalseacucumberspecies,suchas*Holothuriascabra*(81.66%),*Holothuriaspinifera*(80.48%) *Bohadschiasp.*(86.48%),*Bohadschiamarmorata*(84.65%)(Nishanthan*etal.*,2018)and*Holothuria leucospilota*(84.52%)[18].Themoisturecontentcanbeinfluencedwithenvironmental,geographical variations,behaviour,feedingandthecollectiontimeoftheyear[3].However,relativelylargeamount ofmoistureindicatesthepotentialityofconcentratingthenutrientsthroughlossofwateror dehydrationwhichis importanttoincrease theshelf-life whencommercializing[19] .

Ashcontentmaydependonthemineraldepositandotherorganicmatterinfleshwhichget influencedbytheenvironmentandspecies[20].The ashcontentof *H.atra*flesh wasmeasured as 2.5%indicatingrelativelylowashcontentthanotherseacucumberspeciesreportedsuchas, *Holothuriatubulosa*(5.13%),*Holothuriapolii*(7.85%)and*Holothuriamammata*(5.13%)byAydınetal [21].

Theproteincontentisimportantforqualityandtextureofthemuscleofaquaticanimals.Theprotein contentofthe*H.atra*fleshwasrecordedas10.2%basedontheweightindicatingahighervalue comparedtootherspeciesas*H.polii*(8.66±1.2%),*H.tubulosa*(8.82±0.30%)and*H.mammata* (7.88±0.3%) byAydınetal.[21].

Itwasobservedthatbodywallof*H.atra*has0.2%crudefibreand2.0%fatcontentshowsthatsea cucumberstendtohavelowcontentoflipid.Thefatcontentofseacucumbersmightbeinfluenced with several factors such as species, reproductivity, feed availability, feeding pattern and environmentalconditionswhileittendstobehigherinconstanttemperaturesthanfluctuating temperatures.However,fatisanimportantfactortobeconsiderednutritionallyduetoitsimportance as anenergy sourceandrelevance formany otherimportant functionsin thehuman body[22].

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1 ReportedCarbohydratecontent(2.1%)ofthestudywashigherthanseveralpreviousstudies(0.86%
2 in *Paracaudinaaustralis*)[23], whilethe reportedenergy value of*H.atra*was 67kcal/100g.

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Table1:Proximateanalysis(%wetweightbasis)offleshof*H.atra*

| Parameter | Weight(%) |
|---|---|
| MoistureContent | 83.2 |
| Ash content | 2.5 |
| ProteinContent | 10.2 |
| Crude Fibre Content | 0.2 |
| Fat Content | 2.0 |
| CarbohydrateContent | 2.1 |

6

7

8 **3.2.2 Lipidprofileanalysisof*Holothuriaatra***

9 Seacucumbersarebelievedtocontainbioactivesubstancesthat assistinmanyphysiological
10 processessuchaswoundhealingastheyfeedonbottomsedimentsenrichedwithbranchedchain
11 fattyacids[24].Therefore,thefatty acidscompositionof seacucumbers,especially PUFAwill beof
12 hugeconcerned.Basedontheresearch,totalofsaturatedfattyacid(SFA)ismoreoverinsimilar
13 rangewiththe totalof monosaturatedfatty acid(MUFA) andtotal ofpolyunsaturated fattyacid(PUFA).
14 Asperthepreviousstudies,allmostofseacucumberhaveshownhigheramountofsaturatedfatty
15 acidandMonosaturatedfattyacidandlowercontentofPolyunsaturatedfattyacid[25].Thisresults fit
*16* withRidzwanetal.[25] which showedthat saturatedfatty acids werefound dominated in*H.scabra,H.*
17 *leucospilota*and*H.atra*aswell.Further,therecordedlowEPAvalue(0.05g/100g)andabsenceof
*18* DHAaresimilarlyrecordedinthepreviousstudyof4seacucumberspecies;*S.horrens,H.*
19 *leucospilota,H.atraandH.scabra*[25].

Table2:Fattyacidprofileoffleshof*H.atra*

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| Parameter | Amount |
| --- | --- |
| Saturatedfattyacid(g/100g) | 0.4 |
| Monosaturatedfattyacid(g/100g) | 0.3 |
| Polyunsaturatedfattyacid(g/100g) | 0.1 |
| Eicosapentaenoicacid(EPA)(g/100g) | 0.05 |
| Docosahexaenoicacid(DHA)(g/100g) | Not |
| detectedCholesterolcontent(mg/100g) | 1.1 |

1

2 **3.2.3 Vitamin(VitaminAandC)analysisofSeacucumberflesh**

3 VitaminAandCwerenotdetectedin*H.atra.*Nevertheless,VitC(3.19mg/100g)hasbeendetected
4 in*H.scabra*whichbelongtothesamegenus[26].Nutrient levelsolely dependson environmental
5 factors, season, location,type and sizeof the seacucumber species.

6 **3.3Mineralanalysisof*Holothuriaatra***

7 Evaluationofmineralcompositionof*H.atra*ispresentedintable3.Ithasbeenreportedinvarious
8 studiesthatseacucumbersarealsoasourceofminerals,ingeneral,compositionisinfluencedbya
9 numberoffactorssuchasphysiologicalfactors,environmentalconditions,habitatandlifecycle[27].
10 Sodium(Na)wasthelargestinthecompositionfollowedbycalcium(Ca),magnesium(Mg),
11 potassium(K), zinc (Zn), iron (Fe), copper (Cu), selenium (Se) and manganese (Mn).

12 Table3:Mineralcompositionof*H.atra*flesh

| Mineral | Amount(mg/kg) |
| --- | --- |
| Calcium(Ca) | 1200 |
| Magnesium(Mg) | 1100 |
| Sodium(Na) | 4200 |
| Potassium(K) | 629 |
| Zinc (Zn) | 5.9 |
| Selenium(Se) | 2.1 |
| Copper(Cu) | 4.0 |
| Iron(Fe) | 5.3 |

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| Manganese(Mn) | 1.7 |
|---|---|

1 ### 3.4CalculationofyieldofASC

2 Acid-SolubilizedCollagen(ASC)extractionforthebodywallofseacucumbers(*Holothuriaatra*)
3 resultedawhitecolourcottonwoollikesolidwithayieldof0.95%basedonthedryweightbasis.This
4 ishigherthantheyieldrecorded(0.88%)inthepreviousstudyconductedonthesamespeciesby
5 YuniatiandSulardiono[15].Aloweryieldcan beresulteddueto covalentcrosslinkinginthe peptide
6 region,thusreducingthecollagensolubility,whilethelevelofcollagenyieldcanbeincreasedafter
7 peptidedigestionusingpepsinenzymewhile improvingthecollagenextractionability[28].

8 ### 3.5Physico-chemicalcharacterizationofcollagen

9 #### 3.5.1 Determinationofmoisturecontentandph

10 Themoisturecontentofthecollagenfrom*H.atra*wasmeasuredas7.246%.Theaffinityofcollagen
11 andwaterishighlyaffectedbycollagenorganization[29].Further, thepH valueplays acrucial rolein
12 formulationofcollagen,especiallyforcommercializationpurposes[30].The pH of the *H.atra*in the
13 currentstudywasmeasuredas3.95andthismightbeinfluencedbytheacidconcentrationusedin
14 theextraction.Asimilarresulthasbeenreportedinapreviousstudyby[31],which usedPSC (Pepsin
15 SolubleCollagen)extractionmethod.Asperpreviousstudies,thebestpHrangeofcommercial
16 collagen for cosmeticswas reported between3.8 to4.7 [30].

17 #### 3.5.2 AttenuateTotalReflectance-FourierTransformInfraredSpectroscopy(ATR-FTIR)

*18* TheFTIRspectraforextractedcollagensamplefrom*Holothuriaatra*isillustratedinthefigure1.
19 Majorfunctionalgroupsofcollagenswereidentifiedintheextracted*H.atra*collagensampleand
20 confirmedbythepresenceofcharacteristicbands,amideA(3282.60cm$^{-1}$),amideB(2900-3080cm$^{-1}$),
21 amideI(1631.75cm$^{-1}$),amideII(1330-1545cm$^{-1}$),amideIII(1075-1240cm$^{-1}$).AmideAbandisresulted
22 fromstretchingvibrationsofN-Hgroup.Therange3400to3440cm$^{-1}$iscommonlyattributedtothe
23 N-HstretchingvibrationandwhentheN-Hstretchingisinvolvedwithhydrogenbonds,theamideA
24 peakisshiftedtolowerwavenumber[32].Therefore,theresulted peakofamideAcloseto 3300cm$^{-1}$
25 inthisstudyindicatespresenceofH-bondsintheN-Hstretchingoftheextractedcollagensample.
26 TheasymmetricalshapeoftheamideApeakobservedfurtherrevealspresenceofsomeamountof
27 waterinthecollagensample.AsperSusiandArd[33], absorptionband forboundwater shouldbe
28 overlappedwithamideAillustratingalow-frequencyshiftof~100cm$^{-1}$fortheH$_2$Oabsorptionband.



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TheobservedpeaksofAmideBwereidentifiedandattributedat3070.49cm$^{-1}$forCH$_2$asymmetric stretchingandat2931.85cm$^{-1}$forCH$_2$symmetricstretchingwhichwerereportedby AbeandKrimm [34] inthe similarrange.Thepeakappearing at1631.75 cm$^{-1}$was assignedto amideI bandwhichis associatedwithstretchingvibrationsofthecarbonylgroup(C=Obond)alongthebackboneofthe polypeptidechain.TheamideIpeakisusuallyfoundintherangefrom1600to1700cm$^{-1}$andthe lowersideshiftedfrequencyobservedwasduetothehighnumberofH-bondsofthecarbonylsofan amide group[32].

AmideIIpeakwasobservedat1545.45cm$^{-1}$wasattributedtotheN-Hbendingvibrationstrongly coupledtotheC-Nstretchingvibrationofproteinamidegroupswhichareusuallyobservedinthe range1530-1550cm$^{-1}$[35].AmideII minorbandsat lowerfrequencieswere alsoresultedin thestudy. Thepeakat1449.81cm$^{-1}$wasattributedtoCH$_2$bendingandthepeakat1385.50cm$^{-1}$wasderived fromCOO-symmetricalstretching.Thepeakat1337.94cm$^{-1}$ wasobservedwasattributedtothe waggingvibrationoftheprolinesidechainsfoundinthetypeIcollagenofbodytissues[35].Amide III peaksattributedtotheN-HbendingcoupledwithC-NstretchingandC-Ostretchingweredetectedat 1237.00cm$^{-1}$and1075.82cm$^{-1}$respectively.Moreover,functionalgroupanalysiswithFTIRconfirm thepreservedtriplehelixstructureintheextractedcollagensamplefrom*Holothuriaatra*withamide III/IIratioof0.85whichisapproximatelyequalto1.0.Thesimilarresultswerereportedpreviously for *Stichopusjaponicas*and *Holothuriaparva*[28].

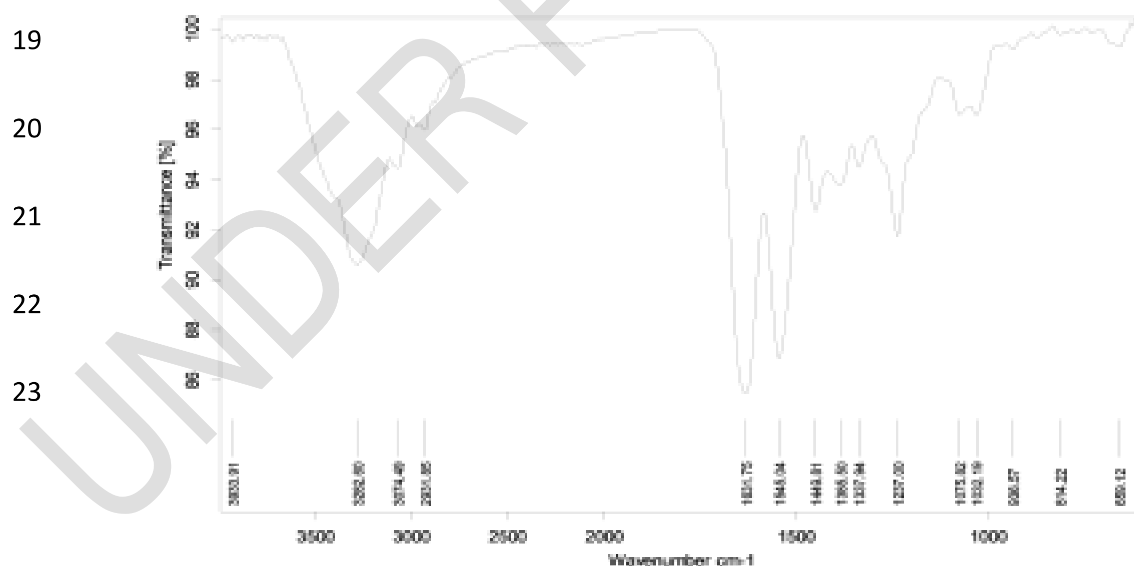


**Fig1**.TheFTIRspectrumofacidsolublecollagensfrom*Holothuriaatra*(ModelThermoScientificNicoletS10FT-IRspectrometer,InstrumentCentre,USJ)



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1 **3.5.3 UV–Visabsorptionspectraanalysis**

2 TheUV-Visspectrophotometricanalysiswasconductedtodetermine collagen'stypeandpuritybased
3 ontheabsorptionatapreidentifiedwavelength.TheUV-Visspectraofextractedcollagenfrom
4 *Holothuriaatra*(Figure2)exhibitedamaximumabsorbanceat232nm.Aspertheobserved
5 maximumabsorbanceat232nm,identifiedthepresenceoftype1collageninthetestedsample.
6 Further,theabsorbancepeaksobservedbetween200-220nmwereattributedtothecollagenpeptide
7 bonds.Thearomaticsidechainscontainedincollagenabsorblightinthe240-300nmregionresulting
8 severalpeaksat280nm(tryptophan),275nm(tyrosine),258nm(histidine,andphenylalanine)[31].
9 However,thenoobviousabsorptionobservedataround250-280nmindicatingthefactthatthe
10 extractedcollagenisinhighpurity.

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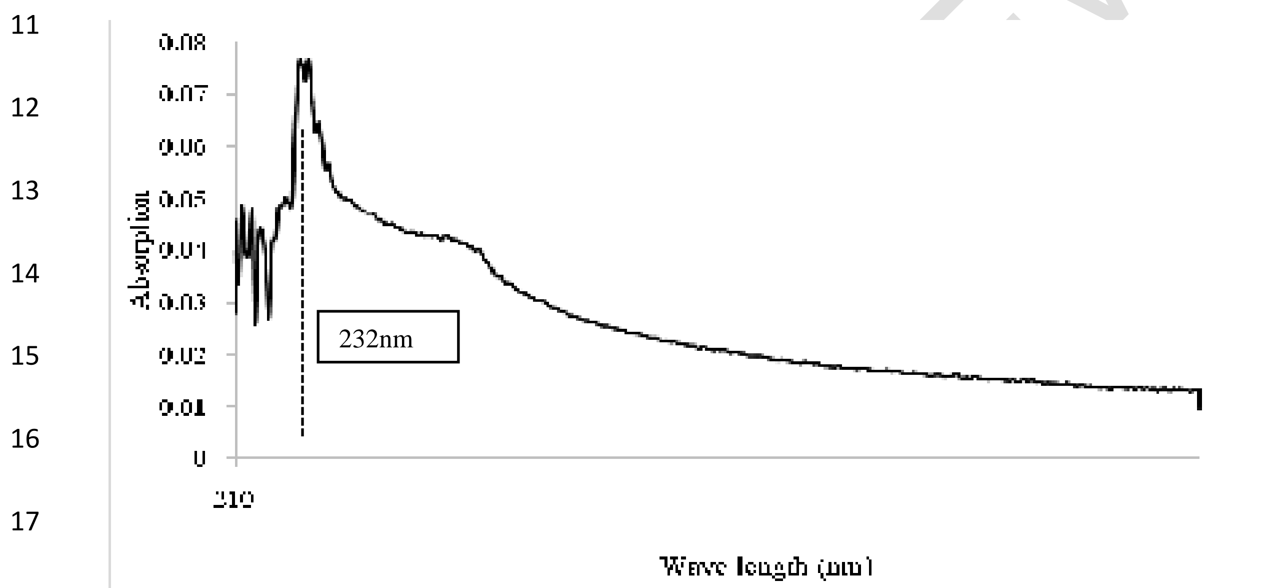


**Fig2.**TheultravioletabsorptionspectrumofACS(ModelThermoScientificGENESYS10SSeriesUV-Visspectrophotometer,InstrumentalCentre,USJ)

19

20 **3.5.4 ScanningElectronMicroscopy(SEM)analysis**

21 Figure3showsthescanningelectronmicroscope(SEM)imagesofcollagensfrom*Holothuriaatra*.It
22 showsbundlesofcircumferencefibrilsnetworks,havingirregularanddensepleatedappearancewith
23 a loose andporous structure whichdescribed by Saallahet al. [31].

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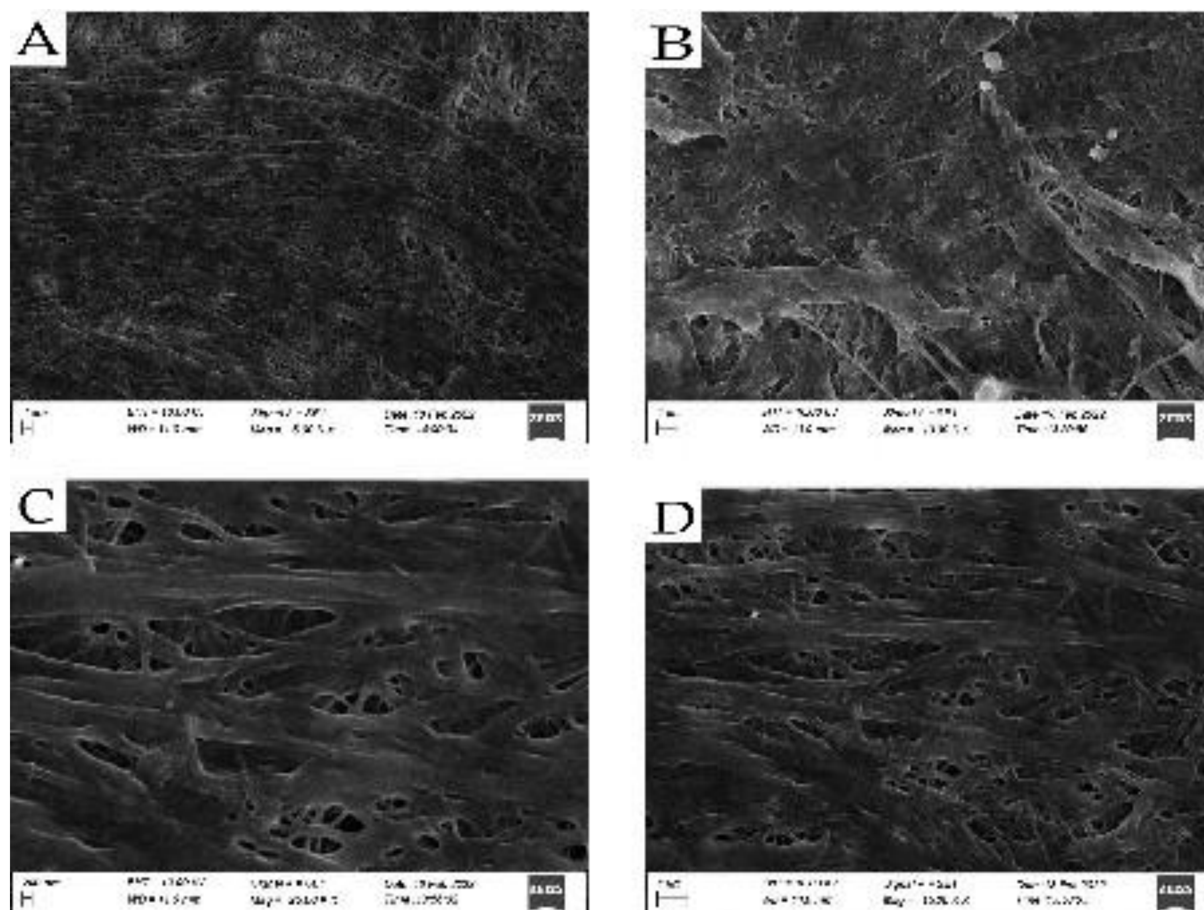

**Fig3.**SEMimagesofASCfrom*Holothuriaatra*.(A)5kx;(B)10kx;(C)25kx(D)15kx(CARLZEISSEVO18ScanningElectronMicroscope,Departmentofmaterialsciences,UniversityofMoratuwa)

## 4.CONCLUSION

Thisstudypresentnutritionprofileandcollagencharacterizationof*Holothuriaatra*bodywallcollected fromNorth-westcoast,SriLankaforthefirsttime.Itisrichwithvariousnutrients.Further,Collagen wasextractedfrom*Holothuriaatra*bodywallusingtheacidbaseextractionmethodwhichcanbe consideredasalowyield.However,thecollagenwascharacterizedastypeIcollagenwithaunique structure.Applicationofcollagenfrom*Holothuriaatra*inindustryisquestionableduetothelowyield thusanalternativeextractionmethodordevelopmentofaproductwithlowyieldisrequiredinthe futuredirections.

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