UDC 639

GENETICS AND AMINO ACID COMPOSITION IN WOTON PLANTS (S*TERCULIA* SP.) FROM RAJA AMPAT: AN ALTERNATIVE NUTRITION MATERIAL FOR FISHES

Supriatna Iman*, **Hismayasari Intanurfemi Bacandra**, **Sayuti Mohammad**, **Budiadnyani I Gusti Ayu**, **Yani Ahmad, Saidin**
Polytechnic of Marine and Fishery, Sorong, West Papua, Indonesia
*Eq-mail: imansupriatna.kkp@gmail.com

ABSTRACT
This study aimed to determined the genetic, phylogenetic, and amino acid composition of the *woton* plant (*Sterculia sp*) from Raja Ampat that could be utilized in improving nutrition, growth, and fish health. The research used bioinformatics analysis through DNA amplification of the samples of *woton* plant (WT01 and WT02) with PCR, homology analysis with BLASTN, phylogenetic with Clustal O (1.2.4) program, and analysis of amino acid composition with Expasy Translate Tool, Proparam, Proscale, and compound bioactive with Prosite. The results showed that WT01 (849 bp) 97% identical and WT02 (875 bp) 99% identical were most homologous alignments with *Sterculia tragacantha* (ID: AY321178.1). WT01 and WT02 had have a 94.21% identity level and were in one group with *Sterculia lanceifolia* (ID: KR531475.1), *Sterculia lanceifolia* (ID: KR531477.1) and *Sterculia brevissima* (ID: KR531473.1). The results used ProtParam that the major amino acid composition in WT01 were 13.9% serine, 12.2% leucine,10% lysine, 7.2% arginine, and 7.2% isoleucine and in WT02 were 12.8% serine, 12.3% leucine, 9.5% lysine, 7.3% arginine, and 7.8% isoleucine. Bioinformatics analysis with Prosite showed that WT01 and WT02 have a small potential of active compound BIG 1(bacterial Ig-like domain 1).

KEY WORDS
Sterculia, woton, genetic, amino acids, bioinformatics.

Raja Ampat Regency is an area rich in natural resources and genetic diversities. *Woton* plant (*Sterculia sp*) is one of the endemic plants of Papua and Raja Ampat that has the potential to help improve nutrition and health. *Sterculia tragacantha* contains anti-inflammatory bioactive compounds, anti-nociceptive, and anti oxidant activities [1]. *Sterculia quinqueloba* has a bioactive compound that can act as anti-bacteria, i.e. *Mycobacteria madagascariense* and *Mycobacteria indicuspranii* [2]. Some researchers have found many benefits for food [3]. *Sterculia setigera Del*. is potential as an herbal plant to treat TB disease in humans [4]. The seeds of *Sterculia urens L.*have been reported to contain 30.88% protein and 39.2% lipid [5]. It is further reported that the major amino acid content in *Sterculia urens L.* is glutamic acid, arginine and aspartic acid, while cysteine, methionine, tyrosine and histidine are observed in small amounts. The role of amino acids is essential in to create equilibrium to improve the efficiency and profitability of global aquaculture production [6]. Research on nutritional composition shows that *Sterculia setigera* is a cheap, nutrient-feeding feedstock needed by fish [7], as well as proper nutrition and formulation requirements when feeding for each species of fish [8]. Amino acids (AA) were traditionally classified as nutritionally essential (EAA) or nonessential (NEAA) for animals and humans based on nitrogen balance or growth [9], but adequate provision of all amino acids (including NEAA) in diets enhances the efficiency of animal production including for mammals, birds and fish [10]. This study aims to determine the genetic diversity and composition of amino acids and bioactive compounds in w*oton* plants (*Sterculia sp*.) from Gag Island, Raja Ampat Regency. The plant has the potential to improve nutrition for fish growth and health. The analysis of DNA barcoding and phylogenetic uses a PCR analysis with matK that characterizes the molecular biology and evolution [11].

MATERIALS AND METHODS OF RESEARCH

Woton plant samples (*Sterculia sp*.) were taken from Gag Island Raja Ampat in July 2017, consisting of two types of woton plants coded as WT01 and WT02. The genomic DNA extraction WT01 and WT02 was done using ZR Plants and Seed DNA MiniPrep™ Kit (Zymo Research). Zymoclean™ Gel DNA Recovery Kit short protocol was added with 3 volumes of ADB Buffer to each volume of gel, incubated at 55ºC for 5-10 minutes (not above 60ºC), then added the melted agarose solution into a *Zymo-Spin™ Columnand*. This was placed into a 2 ml collection tube, centrifuged for 5-10 seconds, and then added with 200 µl of Wash Buffer to the column and span for 10 seconds. Added 200 µl of Wash Buffer and span for 30 seconds then placed the Zymo-Spin Column into a new 1.5 ml tube, added 6 or 10 µl of water directly to the column matrix and span to elute the DNA. PCR amplification was done using KOD FX Neo (Toyobo). PCR products were purified with Zymoclean™ Gel DNA Recovery Kit (Zymo Research) than Bi-directional Sequencing. DNA barcoding was done using PCR with matK as matK gene has two unique features that emprhasize its importance in molecular biology and evolution [9].

The sequencing results of WT01 and WT02 samples were then tested using BLASTN in NCBI to see the homology of DNA samples with NCBI database and phylogenetic tree test with Clustal O (1.2.4) [12,13]. The amino acid (AA) composition was analyzed using bioinformatics analysis by first converting the DNA sequence into an amino acid sequence with Expasy Translate Tool. Then 3'5 'frame sequence is selected compared to BLASTX result in NCBI and defined as ORF sequence (open reading frame) samples of WT01 and WT02 further analyzed for AA composition with ProtParam, ProtScale, and Prosite program.

RESULTS AND DISCUSSION

Samples and genomic DNA extraction. Genomic DNA extraction samples WT01 and WT02 were tested using NanoDrop™ Reading – Genomic DNA, and the results were tested using spectrophotometric A260/280 dan A260/230 for 30 µl as presented in Table 1.

Figure 1 – Woton plant (*Sterculia* sp.): a. *Sterculia* sp. (WT01); b. *Sterculia* sp. (WT02)

Table 1 – Spectrophotometric Results

No	Sample Name	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Volume (µl)
1	WT01	36.5	1.50	1.17	30
2	WT02	107.1	1.52	0.84	30

DNA quality was seen from the absorbance ratio A260/280 (R), while the DNA concentration value was indicated by the concentration (C). Observation of the purity and concentration of DNA results is as shown in table 1. WT01 has a concentration of 36.5 ng/µl and WT02 has a concentration of 107.1 ng/µl.

R values below 1.8 indicate that the DNA samples obtained are stained by proteins, while R values above 2.0 indicate that the DNA samples are still not purified from RNA

impurities. Samples that are still contaminated will complicate the amplification process [14]. From the results of electrophenogram (Fig. 1) and the measurement of concentration value, and the measurement of DNA purity value, it can be considered good enough to be done next process is PCR amplification.

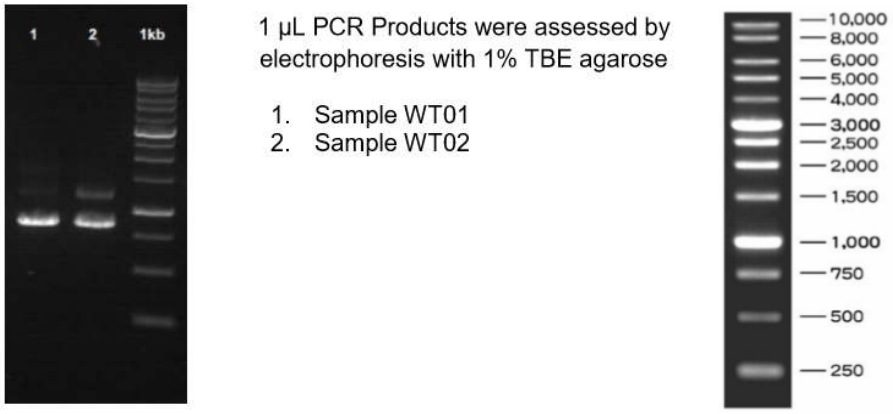


Figure 2 – Gel Photo – PCR Products

Based on the results of PCR amplification and electrophoresis with 1 µL PCR products, assessed by electrophoresis with 1% TBE agarose in WT01 sample and WT02 sample, it was known that the DNA bands of WT01 (1) and WT02 (2) samples were in the position between 750 bp - 1000 bp as is shown in Figure 2. The sequence result of each sample shows that the WT01 sample has a sequence length of 849 bp and the WT02 sample has a sequence length of 875 bp as shown in table 2 below.

Table 2 – Sequences Result

No	Sample Name	Sequences
1	WT01	Assembly of 2 sequences 849 bp 1 TTTTGTGTTT ACGAGCCAAA GTTTTAACAC AAGAAAGCCG AAGTAGATAT TTTATTCGAT 61 ACAAACTTTT TTTTTTTGAA GATCCACTGT GATAATGAGA AAGATTTCTG CATATACGCA 121CAAATCGCTC AATAATATCA GAATCGGAGG AATCGGCCCA CGTCGGCTTA CTAATAGGAT 181GCCCTAATGT GTTACAAAAT TTCGCTTTAG ACAATGATCT ACTAAGAGAA ATAATTGGAA 241 TTCTTGTATC CAACTTCTTC ATAGCATTAT CTATTAGAAA TGAATTTTCT AGCATTTGAC 301 TCCGTACCAA TGAAGGATTT AATCGCACAC TTGAAAGATA GCCCAAAAAG TCGAGAGAAT 361 ATTTAGATAA TTGATTTATA CGGACTCTTC CTGATTGAGA CCACATGTAA AAATAAAAAT 421 AATATTGCCA TAAATCGACA AAGTAATATT TCCACTTATT CATCAGAAGA GACGTATCTT 481 TTGAGGCCAG AATTGCCTTT CCTTGATACC TAATAAAATG TATGAAAGGG TCTTTGAACA 541 TCAATAGGTT GTTCTGAAAA TCATTATAAA AGACTTCTTC AAGATACTCT ATTTTTCCAT 601 AAAAATAAAT GCGTTCAAAA AAGACTCCAG AATATGTTGA TCGTAAATGA GAAGATTGGT 661 TACGGAAAAA AAGCAAAATG GATTCGTATT CACATACATA AGAATTATAT AGGAACAAGA 721 ATAATCTTCG ATTAAAAATC GAAAGAGATT TCTTTGGAGT AAAAAACTCT TCAATTACAT 781 ACTCGTAAAG AGAGAAAGTA TAAATGCAAG AAGAAGATCT TTTACCAGTA GCAAGGGCTT 841 GAACAAGAT

2	WT02	Assembly of 2 sequences 875 bp 1 TGTGTTTACG AGCCAAAGTT TTAACACAAG AAAGCCGAAG TAGATATTTT ATTCGATACA 61 AACTTTTTTT TTTTGAAGAT CCACTGTGAT AATGAGAAAG ATTTCTGCAT ATACGCGCAA 121 ATCGCTCAAT AATATCAGAA TCGGAGGAAT CGGCCCACGT GGGCTTACTA ATAGGATGCC 181 CTAATGTGTT ACAAAATTTC GCTTTAGACA ATGATCTAAT GAGAGAAATA ATTGGAATTC 241 TTGTATCCAA CTTCTTCATA GCATTATCTA TTAGAAATGA ATTTTCTAGC ATTTGACTCC 301 GTACCAATGA AGGATTTAAG CGCACACTTG AAAGATAGCC CAGAAAGCCG AGAGAATATT 361 TATATAATTG ATTTATACGG ACTCTTCCTG ATTGAGACCA CATGTAAAAA TCAAAATAAT 421 ATTGCCATAA ATCGACAAAG TAATATTTCC ACTTATTCAT CAGAAGCGAC GTATCTTTTG 481 AGGCCAGAAT TGCCTTTCCT TGATACCTAA TAAAATGTAT GAAAGGGTCT TTGAACATCC 541 ATAGGTTGTT CTGAAAATCA TTATAAAAGA CTTCGACAAG ATACTCTATT TTTCCATAGA 601 AATAAATGCG TTCAAGAAAG ACTCCAGAAT ATGTTGATCG TAAATGAGAA GATTGGTTAC 661 GGAGAAAAAG CAAAATGGAT TCGTATTCAC ATACATAAGA ATTATATAGG AACAAGAATA 721 ATCTTGGATT AAAAATCGAA ATAGATTTCT TTGGAGTAAG AAAACTCTTC AAATTACAAT 781 ACTCGTAGAG AGAGAAACGT AATAAATGCA AAGAAGAAGC ATCTTTTACC CAGTAGCGAA 841 GGGCTTGAAC CAAGATTTCC AGATGGACTG GGTAA

Sequence alignment and phylogenetics. Phylogenetic analysis of the cyclopropanefatty acid synthase (CPS) family was conducted by using full length protein sequences from cotton and cyclopropanefatty acid synthase from *Sterculia,* full-length amino-acid sequences were first aligned by CLUSTAL O version (1.2.4) with default parameters (http://www.ebi.ac.uk/Tools/clustalw/). Phylogenetic analysis may be considered to be a highly reliable and important bioinformatics tool [15].

Table 3 – BLASTN results WT01 with NCBI database

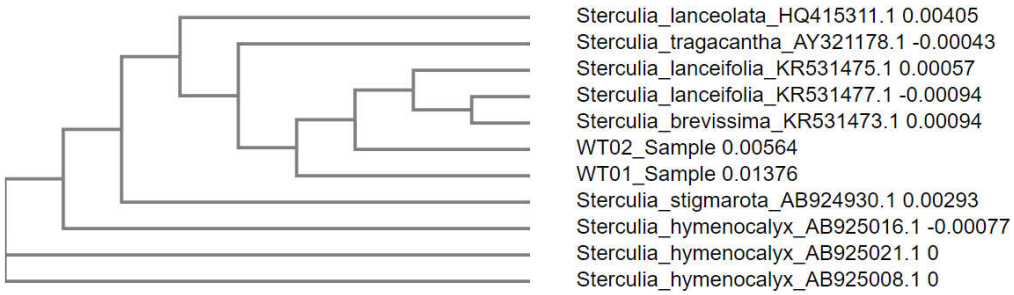
Description	Max score	Total score	Query cover	E value	Ident	Accession
Sterculia tragacantha trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chlc	1439	1439	100%	0.0	97%	AY321178.1
Heritiera littoralis trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloropl	1351	1351	100%	0.0	95%	AY321181.1
Sterculia hymenocalyx chloroplast matK gene for maturase K, partial cds, specimen_voucher: KYUI	1349	1349	94%	0.0	97%	AB925008.1
Tilia americana trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplas	1345	1345	100%	0.0	95%	AY321191.1
Sterculia hymenocalyx chloroplast matK gene for maturase K, partial cds, specimen_voucher: KYUI	1343	1343	94%	0.0	97%	AB925021.1
Tilia amurensis plastid, complete genome	1339	1339	100%	0.0	95%	KT894772.1
Tilia paucicostata plastid, complete genome	1334	1334	100%	0.0	95%	KT894775.1
Tilia oliveri plastid, complete genome	1334	1334	100%	0.0	95%	KT894774.1
Tilia mandshurica plastid, complete genome	1334	1334	100%	0.0	95%	KT894773.1
Cola acuminata trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloropla	1334	1334	100%	0.0	95%	AY321179.1
Patinoa sphaerocarpa maturase K gene, complete cds; chloroplast	1334	1334	100%	0.0	95%	AY589074.1
Ochroma pyramidale trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chlo	1328	1328	100%	0.0	95%	AY321172.1

Sequences alignment was done using BLASTN against NCBI database. BLASTN results against NCBI database excluding uncultured sample sequences showed that WT01 sequence alignment was 97% identical and 100% query cover with *Sterculia tragacantha*

sequence (ID: AY321178.1),95% identical and 100% query cover with *Heritiera littoralis* sequence (ID: AY321181.1), 97% identical and 94% query cover with *Sterculia hymenocalyx* sequence (ID: AB925008.1) (see Table 3). BLASTN results against NCBI database shows that WT02 sequence alignment was 99% identical and 100% query cover with *Sterculia tragacantha* sequence ID: AY321178.1 and 99% identical and 94% query cover with *Sterculia hymenocalyx* sequence ID: AB925021.1(see Table 4).

Table 4 – BLASTN results WT02 with NCBI database

Description	Max score	Total score	Query cover	E value	Ident	Accession
Sterculia tragacantha trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1525	1525	100%	0.0	99%	AY321178.1
Heritiera littoralis trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1443	1443	100%	0.0	97%	AY321181.1
Sterculia hymenocalyx chloroplast matK gene for maturase K, partial cds, specimen_voucher: KYUM<JPN>:479	1442	1442	94%	0.0	99%	AB925021.1
Tilia americana trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1436	1436	99%	0.0	96%	AY321191.1
Cola acuminata trnK gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1434	1434	100%	0.0	96%	AY321179.1
Tilia amurensis plastid, complete genome	1431	1431	99%	0.0	96%	KT894772.1
Tilia paucicostata plastid, complete genome	1427	1427	99%	0.0	96%	KT894775.1
Tilia oliveri plastid, complete genome	1427	1427	99%	0.0	96%	KT894774.1
Tilia mandshurica plastid, complete genome	1427	1427	99%	0.0	96%	KT894773.1
Patinoa sphaerocarpa maturase K gene, complete cds; chloroplast	1427	1427	99%	0.0	96%	AY589074.1



1: Sterculia_lanceolata_HQ415311.1	100.00	99.06	99.38	99.14	99.10	99.21	47.37	46.22	46.68	46.91	46.91
2: Sterculia_stigmarota_AB924930.1	99.06	100.00	99.23	99.35	99.36	99.35	46.87	46.62	46.62	46.87	46.87
3: Sterculia_tragacantha_AY321178.1	99.38	99.23	100.00	99.29	99.26	99.36	48.63	47.40	46.68	47.55	46.81
4: Sterculia_hymenocalyx_AB925021.1	99.14	99.35	99.29	100.00	100.00	100.00	45.99	45.34	45.77	45.99	45.99
5: Sterculia_hymenocalyx_AB925008.1	99.10	99.36	99.26	100.00	100.00	100.00	46.30	45.83	46.06	46.30	46.30
6: Sterculia_hymenocalyx_AB925016.1	99.21	99.35	99.36	100.00	100.00	100.00	46.67	46.42	46.42	46.67	46.67
7: WT01_Sample	47.37	46.87	48.63	45.99	46.30	46.67	100.00	94.21	95.14	96.54	95.27
8: WT02_Sample	46.22	46.62	47.40	45.34	45.83	46.42	94.21	100.00	99.06	99.17	99.20
9: Sterculia_lanceifolia_KR531475.1	46.68	46.62	46.68	45.77	46.06	46.42	95.14	99.06	100.00	99.86	99.87
10: Sterculia_lanceifolia_KR531477.1	46.91	46.87	47.55	45.99	46.30	46.67	96.54	99.17	99.86	100.00	100.00
11: Sterculia_brevissima_KR531473.1	46.91	46.87	46.81	45.99	46.30	46.67	95.27	99.20	99.87	100.00	100.00

Figure 3 – Phylogenetic tree and percent identity matrix by Clustal O (1.2.4)

Based on the phylogenetic by CLUSTAL O (1.2.4), the samples of WT01 and WT02 belong to the same group of *Sterculia lanceifolia* (ID: KR531475.1), *Sterculia lanceifolia* (ID: KR531477.1), and *Sterculia brevissima* (ID: KR531473.1). WT01 and WT02 have an identity level of 94.21%. WT01 has an identity level of 96.54 % with *Sterculia lanceifolia* (ID: KR531477.1) and WT02 has an identity level of 99.20% with *Sterculia* brevissima (ID: KR531473.1) and 99.17% with *Sterculia lanceifolia* (ID: KR531477.1).

*Translation to Amino Acid Sequences.*To find out the amino acid contents in WT01 and WT02, first the DNA sequences were translated into amino acid sequences by using the Expasy Translate Tool program [16]. The DNA sequences of each sequence are:

>WT01_Sample_Open reading frames
3'5' Frame 3
LVQALATGKRSSSCIYTFSLYEYVIEEFFTPKKSLSIFNRRLFLFLYNSYVCEYESILLF

FRNQSSHLRSTYSGVFFERIYFYGKIEYLEEVFYNDFQNNLLMFKDPFIHFIRYQGKAIL
ASKDTSLLMNKWKYYFVDLWQYYFYFYMWSQSGRVRINQLSKYSLDFLGYLSSVRLNPSL
VRSQMLENSFLIDNAMKKLDTRIPIISLSRSLSKAKFCNTLGHPISKPTWADSSDSDIIE
RFVRICRNLSHYHSGSSKKKSLYRIKYLLRLSCVKTLARKHK

>WT02_Sample_Open reading frames
3'5' Frame 2
YPVHLEILVQALRYWVKDASSLHLLRFSLYEYCNLKSFLTPKKSISIFNPRLFLFLYNSY
VCEYESILLFLRNQSSHLRSTYSGVFLERIYFYGKIEYLVEVFYNDFQNNLWMFKDPFIH
FIRYQGKAILASKDTSLLMNKWKYYFVDLWQYYFDFYMWSQSGRVRINQLYKYSLGFLGY
LSSVRLNPSLVRSQMLENSFLIDNAMKKLDTRIPIISLIRSLSKAKFCNTLGHPISKPTW
ADSSDSDIIERFARICRNLSHYHSGSSKKKSLYRIKYLLRLSCVKTLARKH

Based on the result of the translated sample of WT01 with Expasy Translate Tool and BLASX in NCBI, the translated result on Open Frame is highlighted in red from 3`5` Frame 3 with amino acid sequences arrangement as follows:

M F K D P F I H F I R Y Q G K A I L A S K D T S L L M N K W K Y Y F V D L W Q Y Y F Y F Y M W S
Q S G R V R I N Q L S K Y S L D F L G Y L S S V R L N P S L V R S Q Met L E N S F L I D N A Met
K K L D T R I P I I S L S R S L S K A K F C N T L G H P I S K P T W A D S S D S D I I E R F V R I C
R N L S H Y H S G S S K K K S L Y R I K Y L L R L S C V K T L A R K H K

For the translated sample of WT02 with Expasy Translate Tool and BLASX in NCBI, the translated result on Open Frame is highlighted in red from 3`5` Frame 3 with amino acid sequences arrangement as follows:

M F K D P F I H F I R Y Q G K A I L A S K D T S L L M N K W K Y Y F V D L W Q Y Y F D F Y M W S
Q S G R V R I N Q L Y K Y S L G F L G Y L S S V R L N P S L V R S Q M L E N S F L I D N A M K K
L D T R I P I I S L I R S L S K A K F C N T L G H P I S K P T W A D S S D S D I I E R F A R I C R N L
S H Y H S G S S K K K S L Y R I K Y L L R L S C V K T L A R K H

Amino Acid Composition. Amino acid composition was analyzed using ProtParam program and the results showed that WT01 sample with 180 Amino Acids (AA) and WT02 sample with 178 AA whose compositions are shown in Table 5.

Table 5 – Amino Acid Composition

No	Amino Acid Composition	Samples	
		WT01 Sample_ORF (%)	WT02 Sample_ORF (%)
1	Alanine (A) (NEAA)	3.3	3.9
2	Arginine (R) (EAA)	7.2	7.3
3	Asparagine (N) (NEAA)	3.9	3.9
4	Aspartic acid (D) (NEAA)	5.0	5.0
5	Cystine (C) (NEAA)	1.7	1.7
6	Glutamine (Q) (NEAA)	2.8	2.8
7	Glutamic acid (E) (NEAA)	1.1	1.1
8	Glycine (G) (NEAA)	2.8	3.4
9	Histidine (H) (EAA)	2.8	2.8
10	Isoleusine (I) (EAA)	7.2	7.8
11	Leucine (L) (EAA)	12.2	12.3
12	Lysine (K) (EAA)	10.0	9.5
13	Methionine (M)(EAA)	2.8	2.8
14	Phenylalanine (F) (EAA)	5.6	5.6
15	Proline (P) (NEAA)	2.8	2.8
16	Serine (S) (NEAA)	13.9	12.8
17	Threonine (T) (EAA)	2.8	2.8
18	Tryptophan (W)(EAA)	2.2	2.2
19	Tyrosine (Y) (NEAA)	6.7	6.7
20	Valine (V) (EAA)	3.3	2.8

Notes: EAA= Essential Amino Acid; NEAA= Nonessential Amino Acid.

Based on the results of the analysis using the ProtParam, it is known that the main amino acid composition in the sample of WT01 ORF is 13.9% serine, 12.2% leucine 10% lysine,7.2% arginine, and 7.2% isoleucine. There were slight differences in the main amino acid composition in WT02 ORF samples i.e. 12.8% serine, 12.3% leucine, 9.5% lysine, 7.3% arginine, and 7.8% isoleucine.

This suggests that extract of *woton* plant have the potential to supply the amino acids needed by fish in the report on the amino acid composition present in fish and shrimp feeds that the major essential amino acid in all of the feeds were lysine (2.2 to 3.7%), leucine (2.5 to 3.6%), and arginine (2.4 to 3.4 %) [17].

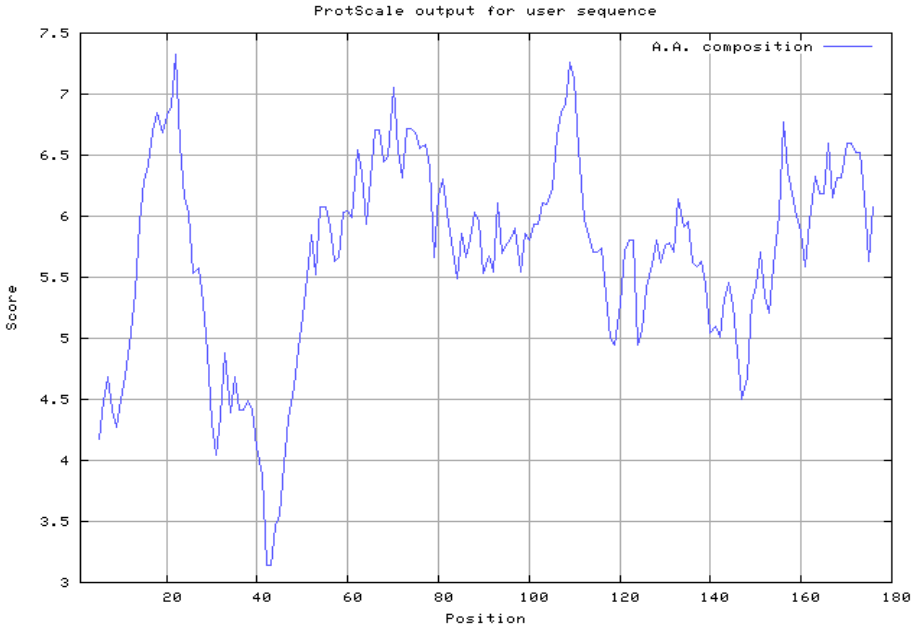


Figure 4 – Proscale output for WT01 and WT02

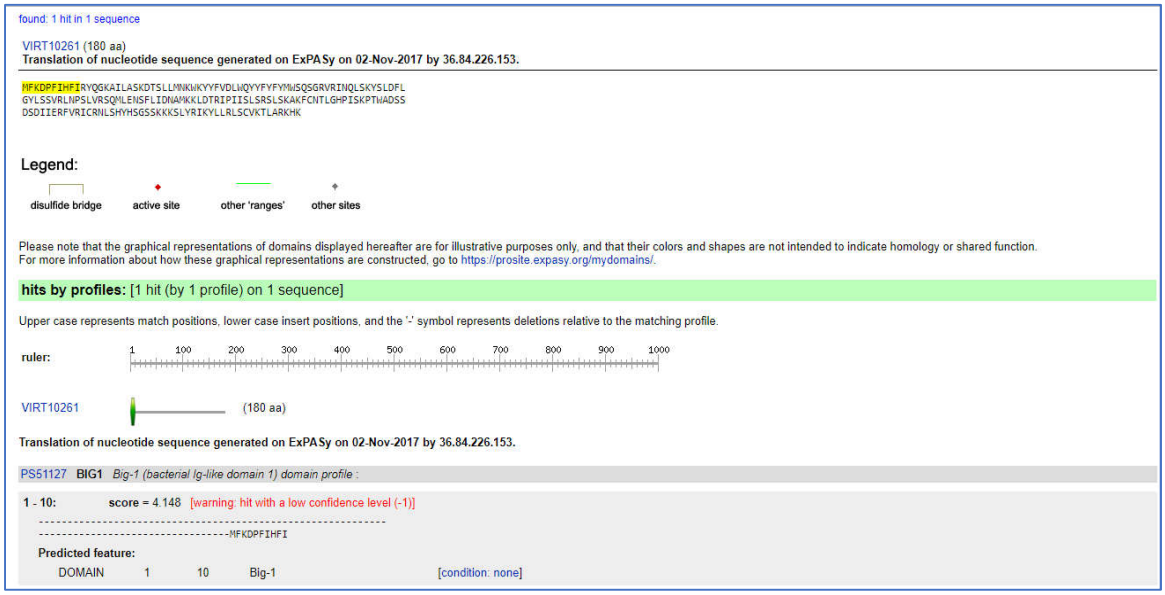


Figure 5 – Prosite result for WT01 and WT02
(Source:http://prosite.expasy.org/cgibin/prosite/ScanView.cgi?scanfile=466868712144.scan.gz)

Over 200 amino acids occur in nature, however, only about 20 of these are considered common. Fish cannot themselves synthesize the 10 indispensable amino acids, so these amino acids must be supplied by the diet. These amino acids are methionine, arginine,

threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Lysine and methionine are often the first limiting amino acids. Based on the ProtScale, it can be seen that tryptophan in the WT01 sample is at a low value of 1.080 and leusine of 9.660.

The bioinformatics analysis with the Prosite showed that WT01 and WT02 have an incomplete sequence of BIG1 (bacterial Ig-like domain 1) active compound which is a family of immunoglobulin superfamily and has been known to have new functional activity where IgG can form stable, non-immune with anaphylatoxin [18]. These phage Ig-like domains fall into three classic immunoglobulin domains (I-Set), the fibronectin type 3 repeat (FN3), and the bacterial Ig-like domain (Big2) [19].

CONCLUSION

Based on the results of analysis and the findings, it can be concluded bioinformatics analysis of sequences WT01 and WT02 are *woton* plant species (*Sterculia sp*.) which have a number of important amino acids and potential active compounds that need further testing for the utilization of nutrition improvements for growth, reproduction, and health of fish.

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