LAMPIRAN

Lampiran 1. Hasil Determinasi:

INSTITUT TEKNOLOGI BANDUNG
SEKOLAH ILMU DAN TEKNOLOGI HAYATI
Jalan Ganesa 10 Bandung 40132, Telp.: (022) 251 1575, 250 0258, Fax.: (022) 253 4107
e-mail: sith@itb.ac.id http://www.sith.itb.ac.id

Nomor : 6543/IT1.C11.2/TA.00/2021 13 Desember 2021
Hal : Determinasi tumbuhan

Kepada Yth.
Dekan
Fakultas Farmasi
Universitas Bhakti Kencana
Jalan Soekarno Hatta No. 754
Bandung

Memperhatikan surat permintaan Saudara dalam surat No. 1288/03.FF/UBK/XI/2021 tanggal 29 November 2021 mengenai determinasi tumbuhan, dengan ini kami sampaikan bahwa setelah dilakukan determinasi oleh staf kami, sampel tumbuhan yang dikirim oleh Sdr. Desti Astriyani (NPM: 11181198), adalah :

No	Nama sampel	Hasil determinasi	Famili
1	Pegagan	*Centella asiatica* (L.) Urb.	Apiaceae

Referensi:

1. Backer, C.A., & Bakhuizen van den Brink, R.C. (1965). *Flora of Java*. Vol. II. Groningen, The Netherlands: NVP Noordhoff. pp. 173.

Demikian yang kami sampaikan. Atas perhatian dan kerjasama yang diberikan, kami ucapkan terima kasih.

Wakil Dekan Bidang Sumber Daya,

Dr. Angga Dwiartama
NIP. 198302052012121002

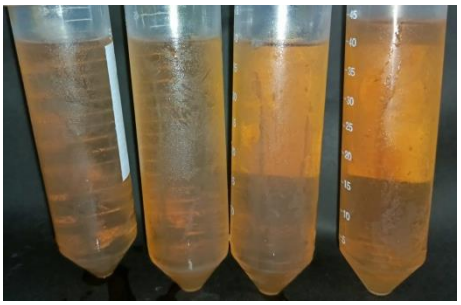
Tembusan:
Dekan SITH ITB, sebagai laporan.

Lampiran 2. Tabel penambahan ammonium sulfat

Initial concentration of ammonium sulfate (percentage saturation at 0 °C)	Percentage saturation at 0 °C															
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (g) to be added to 1 l of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	536	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0

Lampiran 3. Hasil Ekstraksi dan Isolasi

Ekstraksi Pegagan

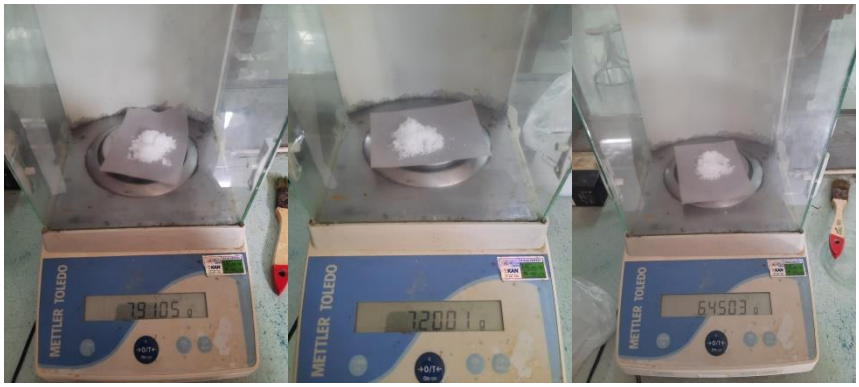


Isolasi dengan cara Sentrifugasi

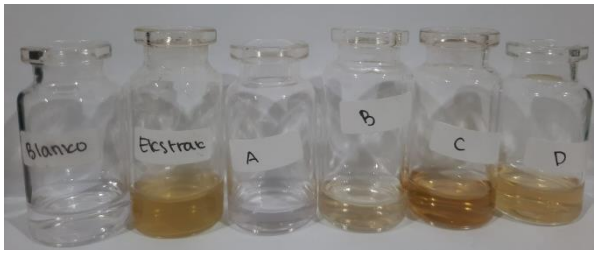
Saat sentrifugasi

dalam alat sentrifugasi

Lampiran 4. Penimbangan ammonium sulfat



Lampiran 5. Hasil uji dan perhitungan aktivitas enzim menggunakan Spektrofotometer

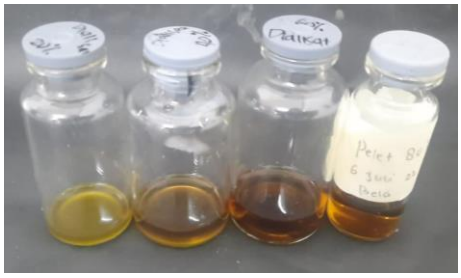


Konsentrasi sampel	ABSORBANSI	RATA-RATA	n	aktivitas SOD
blanko	0.00081	0.00087		
	0.00093			
20	0.15852			
	0.15967	0.159095		181.87
40	0.23160			
	0.23051	0.231055		264.58
60	0.31143			
	0.31053	0.310980		356.45
80	0.24062			
	0.24064	0.240630	1	275.59

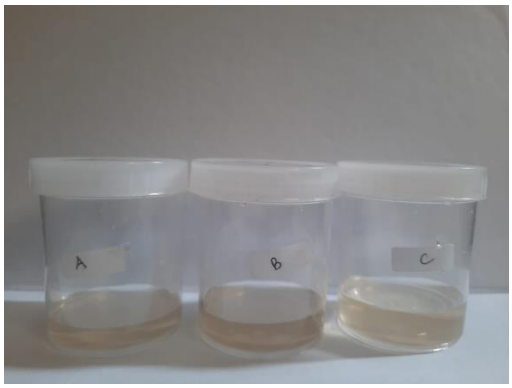
Lampiran 6. Perhitungan DPPH

SAMPEL	KONSENTRASI (µg/ml)	ABSORBANSI KONTROL	ABSORBANSI SAMPEL	% INHIBISI	RATA-RATA	DEVIASI (±)
DPPH	60	0.7517			0.7519	
		0.7520				
b20	20	0.752	0.447	40.61710334		
		0.752	0.447	40.51070621	40.56390477	0.0532
	40	0.752	0.433	42.47905307		
		0.752	0.433	42.45245378	42.46575342	0.0133
	60	0.752	0.374	50.28594228		
		0.752	0.373	50.33914084	50.31254156	0.0266
	80	0.752	0.321	57.30815268		
		0.752	#####	57.42784945	57.36800106	0.0598

Lampiran 7. Hasil Dialisis



Lampian 8. Hasil Formulasi



Lampiran 9. *Certificate of analysis*

a. Buffer pH 4.00.

Supelco®

www.sigmaaldrich.com

Certificate of Analysis – Certified Reference Material

Certipur® Buffer solution pH 4.00 (20°C)
Certified Reference Material for pH measurement

Product no.:	1.09435.1000
Lot no.:	HC01392235
Description of CRM:	Certipur® Buffer solution pH 4.00 (20°C) Certified Reference Material for pH measurement
Minimum shelf life:	2023/01/31
Storage:	+15°C to +25°C tightly closed in the original container
Composition:	citric acid / sodium hydroxide / hydrogen chloride

Certified value	Associated uncertainty, $U=k \cdot u$ ($k=2$)
pH value 4.01	**± 0.02 (20°C)**

Metrological traceability:	The pH value of this certified buffer solution is directly traceable to primary certified reference materials characterised by PTB and verified by SRMs from NIST. NIST 189c, 188, 185i, 186 Ig, 186 IIg, 187f PTB OX-405/18, TA-442/19, PHT-340/16, PHO-346/16, BO-373/17 *PTB: Physikalisch Technische Bundesanstalt, Braunschweig, Germany* *NIST: National Institute of Standards and Technology, Gaithersburg, USA.*
Measurement method:	pH value is measured with a combined glass electrode after 5-point calibration according to DIN 19268 with reference buffer solutions according to DIN 19266, IUPAC, NIST, Ph.Eur. and USP.
Accreditation:	Merck KGaA, Darmstadt, Germany is accredited by the German accreditation authority DAkkS as registered reference material producer D-RM-15185-01-00 in accordance with ISO 17034 and registered calibration laboratory D-K-15185-01-00 according to DIN EN ISO/IEC 17025.
Certificate issue date:	2020/01/22

DAkkS Deutsche Akkreditierungsstelle D-RM-15185-01-00
ISO 17034

DAkkS Deutsche Akkreditierungsstelle D-K-15185-01-00
ISO/IEC 17025

A. Yildirim
Dipl.-Ing. Ayfer Yildirim
(Responsible QC Laboratory Manager)

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EMD Millipore Corporation, 400 Summit Drive Burlington MA 01803, USA, Tel. +1-978-715-4321
Sigma-Aldrich Canada Co. or Millipore (Canada) Ltd., 2149 Winston Park, Dr. Oakville, Ontario, L6H 6J8, Phone: +1 800-565-1400

Certificate Page 1 of 2 Certificate version 02

b. Buffer pH 7.00.

Supelco®

www.sigmaaldrich.com

Certificate of Analysis – Certified Reference Material

Certipur® Buffer solution pH 7.00 (20°C)
Certified Reference Material for pH measurement

Product no.:	1.09439.1000
Lot no.:	HC16190739
Description of CRM:	Certipur® Buffer solution pH 7.00 (20°C) Certified Reference Material for pH measurement
Expiry date:	2024/05/31
Storage:	+15°C to +25°C tightly closed in the original container
Composition:	di-sodium hydrogen phosphate / potassium dihydrogen phosphate

Certified value	Associated uncertainty, $U=k \cdot u$ ($k=2$)
pH value 7.00	**± 0.02 (20°C)**

Metrological traceability:	The pH value of this certified buffer solution is directly traceable to primary certified reference materials characterised by PTB and verified by SRMs from NIST. NIST 189c, 188, 185i, 186 Ig, 186 IIg, 187f PTB OX-405/18, TA-442/19, PHT-467/20, PHO-490/20, BO-468/20 *PTB: Physikalisch Technische Bundesanstalt, Braunschweig, Germany* *NIST: National Institute of Standards and Technology, Gaithersburg, USA.*
Measurement method:	pH value is measured with a combined glass electrode after 5-point calibration according to DIN 19268 with reference buffer solutions according to DIN 19266, IUPAC, NIST, Ph.Eur. and USP.
Accreditation:	Merck KGaA, Darmstadt, Germany is accredited by the German accreditation authority DAkkS as registered reference material producer D-RM-15185-01-00 in accordance with ISO 17034.
Certificate issue date:	2021/05/10

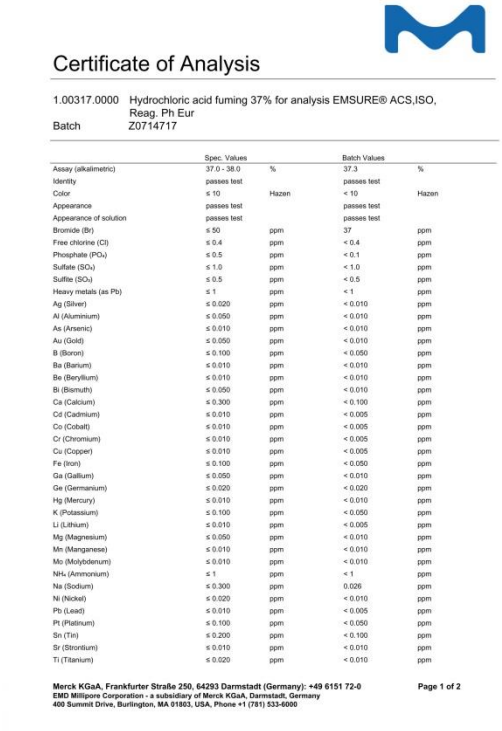
DAkkS Deutsche Akkreditierungsstelle D-RM-15185-01-00
ISO 17034

CRM released by Approving Officer or delegate LS-OII-QS3
A. Yildirim
Dipl.-Ing. Ayfer Yildirim
Responsible Manager of LS-OII-QS3
(Accredited Calibration Laboratory)

Merck KGaA, 64271 Darmstadt, Germany, Tel.: +49 (0)6151 72-2440
EMD Millipore Corporation, 400 Summit Drive Burlington MA 01803, USA, Tel. +1-978-715-4321
Sigma-Aldrich Canada Co. or Millipore (Canada) Ltd., 2149 Winston Park, Dr. Oakville, Ontario, L6H 6J8, Phone: +1 800-565-1400

Certificate Page 1 of 2 Certificate version 01

c. Asam Klorida.



Certificate of Analysis

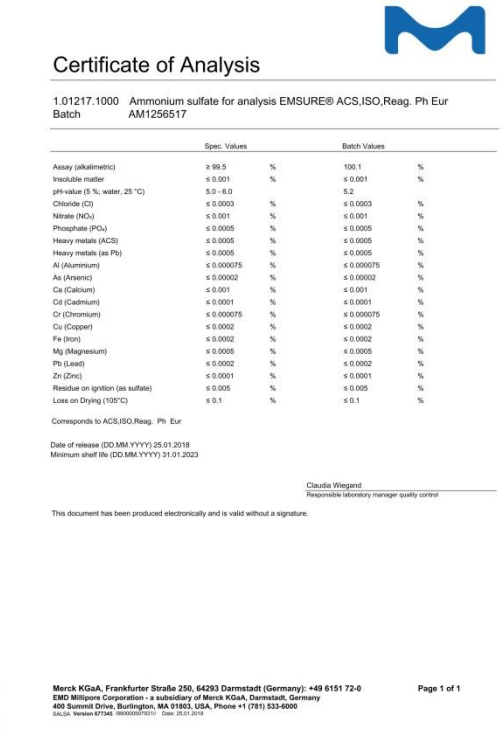
1.00317.0000 Hydrochloric acid fuming 37% for analysis EMSURE® ACS,ISO, Reag. Ph Eur
Batch Z0714717

	Spec. Values		Batch Values	
Assay (alkalimetric)	37.0 - 38.0	%	37.3	%
Identity	passes test		passes test	
Color	≤ 10	Hazen	< 10	Hazen
Appearance	passes test		passes test	
Appearance of solution	passes test		passes test	
Bromide (Br)	≤ 50	ppm	37	ppm
Free chlorine (Cl)	≤ 0.4	ppm	< 0.4	ppm
Phosphate (PO_4)	≤ 0.5	ppm	< 0.1	ppm
Sulfate (SO_4)	≤ 1.0	ppm	< 1.0	ppm
Sulfite (SO_3)	≤ 0.5	ppm	< 0.5	ppm
Heavy metals (as Pb)	≤ 1	ppm	< 1	ppm
Ag (Silver)	≤ 0.020	ppm	< 0.010	ppm
Al (Aluminium)	≤ 0.050	ppm	< 0.010	ppm
As (Arsenic)	≤ 0.010	ppm	< 0.010	ppm
Au (Gold)	≤ 0.050	ppm	< 0.010	ppm
B (Boron)	≤ 0.100	ppm	< 0.050	ppm
Ba (Barium)	≤ 0.010	ppm	< 0.010	ppm
Be (Beryllium)	≤ 0.010	ppm	< 0.010	ppm
Bi (Bismuth)	≤ 0.050	ppm	< 0.010	ppm
Ca (Calcium)	≤ 0.300	ppm	< 0.100	ppm
Cd (Cadmium)	≤ 0.010	ppm	< 0.005	ppm
Co (Cobalt)	≤ 0.010	ppm	< 0.005	ppm
Cr (Chromium)	≤ 0.010	ppm	< 0.005	ppm
Cu (Copper)	≤ 0.010	ppm	< 0.005	ppm
Fe (Iron)	≤ 0.100	ppm	< 0.050	ppm
Ga (Gallium)	≤ 0.050	ppm	< 0.010	ppm
Ge (Germanium)	≤ 0.020	ppm	< 0.020	ppm
Hg (Mercury)	≤ 0.010	ppm	< 0.010	ppm
K (Potassium)	≤ 0.100	ppm	< 0.050	ppm
Li (Lithium)	≤ 0.010	ppm	< 0.005	ppm
Mg (Magnesium)	≤ 0.050	ppm	< 0.010	ppm
Mn (Manganese)	≤ 0.010	ppm	< 0.010	ppm
Mo (Molybdenum)	≤ 0.010	ppm	< 0.010	ppm
NH_4 (Ammonium)	≤ 1	ppm	< 1	ppm
Na (Sodium)	≤ 0.300	ppm	0.026	ppm
Ni (Nickel)	≤ 0.020	ppm	< 0.010	ppm
Pb (Lead)	≤ 0.010	ppm	< 0.005	ppm
Pt (Platinum)	≤ 0.100	ppm	< 0.050	ppm
Sn (Tin)	≤ 0.200	ppm	< 0.100	ppm
Sr (Strontium)	≤ 0.010	ppm	< 0.010	ppm
Ti (Titanium)	≤ 0.020	ppm	< 0.010	ppm

Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt (Germany): +49 6151 72-0
EMD Millipore Corporation - a subsidiary of Merck KGaA, Darmstadt, Germany
400 Summit Drive, Burlington, MA 01803, USA, Phone +1 (781) 533-6000

Page 1 of 2

d. Ammonium sulfate.



Certificate of Analysis

1.01217.1000 Ammonium sulfate for analysis EMSURE® ACS,ISO,Reag. Ph Eur
Batch AM1256517

	Spec. Values		Batch Values	
Assay (alkalimetric)	≥ 99.5	%	100.1	%
Insoluble matter	≤ 0.001	%	≤ 0.001	%
pH-value (5 %; water, 25 °C)	5.0 - 6.0		5.2	
Chloride (Cl)	≤ 0.0003	%	≤ 0.0003	%
Nitrate (NO_3)	≤ 0.001	%	≤ 0.001	%
Phosphate (PO_4)	≤ 0.0005	%	≤ 0.0005	%
Heavy metals (ACS)	≤ 0.0005	%	≤ 0.0005	%
Heavy metals (as Pb)	≤ 0.0005	%	≤ 0.0005	%
Al (Aluminium)	≤ 0.000075	%	≤ 0.000075	%
As (Arsenic)	≤ 0.00002	%	≤ 0.00002	%
Ca (Calcium)	≤ 0.001	%	≤ 0.001	%
Cd (Cadmium)	≤ 0.0001	%	≤ 0.0001	%
Cr (Chromium)	≤ 0.000075	%	≤ 0.000075	%
Cu (Copper)	≤ 0.0002	%	≤ 0.0002	%
Fe (Iron)	≤ 0.0002	%	≤ 0.0002	%
Mg (Magnesium)	≤ 0.0005	%	≤ 0.0005	%
Pb (Lead)	≤ 0.0002	%	≤ 0.0002	%
Zn (Zinc)	≤ 0.0001	%	≤ 0.0001	%
Residue on ignition (as sulfate)	≤ 0.005	%	≤ 0.005	%
Loss on Drying (105°C)	≤ 0.1	%	≤ 0.1	%

Corresponds to ACS,ISO,Reag. Ph Eur

Date of release (DD.MM.YYYY) 25.01.2018
Minimum shelf life (DD.MM.YYYY) 31.01.2023

Claudia Wiegand
Responsible laboratory manager quality control

This document has been produced electronically and is valid without a signature.

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EMD Millipore Corporation - a subsidiary of Merck KGaA, Darmstadt, Germany
400 Summit Drive, Burlington, MA 01803, USA, Phone +1 (781) 533-6000
SALSA **Version 677345** 99000035078310 Date: 25.01.2018

Page 1 of 1

Lampiran 10. Preparasi Reagen SDS-PAGE

a. Larutan Stok Bis-Acrylamide (30% T;2,67%C)

29,2 gram akrilamid dilarutkan dalam100 mL air deionisasi, kemudian ditambahkan 0.78 gram bisacrilamide dan 10% SDS atau 10 gram kedalam larutan aduk hingga homogen menggunakan magnetik stirrer, lalu larutan disaring dan disimpan pada suhu 4ºC ditaruh pada botol coklat atau botol bening yang dilapisi oleh alumuniumfoil.

b. Buffer Separating (1.5M Tris-HCL pH 8.8)

18.15 gram tris base, tambahkan 70 mL air deionisasi dan pH di just hingga 8.8 menggunakan HCL. Jika pH sudah 8.8 tambahkan air deionisasi hingga 100 mL**.**

c. Buffer Stacking (0.5 M Tris-HCl pH6.8)

6.05 gram tris base ditambahkanair sebanyak 70 mL air deionisasi lalu just pH hingga 6.8 menggunakan HCl sedikit demi sedikit lalu jika pH telah 6.8 tambahkan air hingga 100 mL

d. 5x Loading Buffer

15,15 gram base tris 72 gramglisin dan 5 gram SDS dilarutkan dalam 100 mL air deinosasi, larutan diaduk kemudian ditambahkan air deionisasi hingga volume total 1000 mL. larutan disimpan pada suhu 4ºC dan dihangatkan hingga suhu ruang sebelum digunakan.

e. Running Buffer

3 gram tris base di tambahkan dengan 14.4 gram glisin dan 1 gram SDS lalu just hingga satu litter menggunakan air deionisasi hingga pH 8.3

f. 10% APS

1 gram Ammonium persulfate dalam 10 mL air deionisasi

02.66.00/FRM-03/AKD-SPMI 48

Lampiran 1: Format Surat Pernyataan Bebas Plagiasi

SURAT PERNYATAAN

Yang bertanda tangan di bawah ini,

Nama : Salsabila

N P M : 11181222

adalah mahasiswa Fakultas Farmasi, Universitas Bhakti Kencana, menyatakan dengan sesungguhnya bahwa Naskah Tugas Akhir/Skripsi yang saya tulis dengan judul:

Isolasi, Purifikasi, Uji Aktivitas dan Formulasi Serum Superoksida Dismutase dari Daun Pegagan (*Centella asiatica*(L.)) sebagai Anti Radikal Bebas

adalah benar-benar merupakan hasil karya saya sendiri. Apabila di kemudian hari diketahui bahwa isi Naskah Skripsi ini merupakan hasil plagiasi, maka saya bersedia menerima sanksi berupa pembatalan kelulusan dan atau pencabutan gelar yang saya peroleh.

Demikian surat pernyataan ini saya buat untuk dapat dipergunakan sebagaimana mestinya.

Bandung , 05 Agustus 2022

Yang membuat pernyataan,

(Salsabila)

NPM 11181222

02.66.00/FRM-03/AKD-SPMI 49

Lampiran 2: Format Surat Persetujuan untuk dipublikasikan di media on line

SURAT PERNYATAAN

Yang bertanda tangan di bawah ini,

Nama : Salsabila

N P M : 11181222

Menyatakan bahwa demi kepentingan perkembangan ilmu pengetahuan,

Isolasi, Purifikasi, Uji Aktivitas dan Formulasi Serum Superoksida Dismutase dari Daun Pegagan (*Centella asiatica*(L.)) sebagai Anti Radikal Bebas

saya menyetujui abstrak Skripsi yang saya tulis dengan judul

Untuk dipublikasikan atau ditampilkan di internet atau media lain yaitu Digital Library Perpustakaan Universitas Bhakti Kencanauntuk kepentingan akademik, sebatas sesuai dengan Undang-Undang Hak Cipta.

Demikian surat pernyataan ini saya buat untuk dapat dipergunakan sebagaimana mestinya

Bandung ,05 Agustus 2022

Yang membuat pernyataan,

(Salsabila)

NPM .11181222