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Optimization of *Spirulina Platensis* Culture for Antioxidant Production

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ABSTRACT

Spirulina platensis is a unicellular microalgae that has high nutritional value such as proteins, carbohydrates, lipids and carotenoids like β-carotene. The microalgae rapidly grows in Bold Bassal Medium modification and high intensity of light. The aims of this research were to optimize the culture growth and determine antioxidant activity of S. platensis. Culture was optimized by adding urea and $NaHCO_3$ *I nto the medium and applying dark : light period (12:12 h/h) under solar or fluorescent light (3000 lux). The optimum growth of Spirulina platensis was in 0,3 g/L urea, pH 9 at fluoresense light (3000 lux). Antioxidant acivity of various spirulina extract was studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method. The Spirulina radicals scavenging activity of acetone : methanol (1:1) extract was 46,39 %. The acetone:methanol (1:1) extract showed better antioxidant activity than acetone extract. The β-carotene was determined using HPLC in a silica gel column with dichloromethane, acetonitrile, methanol (20:70:10, v/v/v) as a mobile phase. The acetone extract displayed the highest sample area and produced 1.32% of β-carotene out of total biomass.*

Keywords : *Spirulina platensis*, antioxidant, free radical, HPLC, DPPH

INTRODUCTION

Free radicals area highly unstable molecule that containing one or more unpaired electrons in atomic or molecular orbitals [1]. Free radicals also known as reactive oxygen species [ROS] or reactive nitrogen species [RNS]. ROS may lead to stimulation of inflammatory process, secretion of chemotactic factors, growth factors, proteolytic enzymes, lipoxygenases, and cyclooxygenases, inactivation of antiproteolytic enzymes and activation of oncogenes and transcription factors [2]. Free radicals may come from cigarette smoke, air pollution, and sunlight that can causes “oxidative stress” a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease, and eye diseases such as cataracts and age-related macular degeneration [3-7].

Proteins are the major targets for ROS because of their high overall abundance in biological systems. Since proteins are primarily responsible for most of cell activities, their peroxidative damage by ROS is particular importance. It has been estimated that proteins can scavenge the majority [50%–75%] of reactive species generated [8].In order to reduce free radicals damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly used of synthetic antioxidant has been suspected of being responsible for liver damage and carcinogenesis [2]. This is essential to develop and utilize effective and natural antioxidants that can protect the human body from free radicals and retard the progress of many chronic diseases.

To protect cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals [9].

These components include:

1. Nutrient-derived antioxidants like carotenoids including β-carotene, ascorbic acid (vitamin C).
2. Tocopherols and tocotrienols (vitamin E), and other low molecular weight compounds such as glutathione and lipoic acid.
3. Antioxidant enzymes, e.g.superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radicals quenching reactions.
4. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
5. Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Vitamin C, vitamin E, and β-carotene are among the most widely studied dietary antioxidants [10]. β-carotene and other carotenoids are believed to provide antioxidant protection to lipid-rich tissues. Research suggests β-carotene may work synergistically with vitamin E [11; 12]. β-carotene accounts for 80 % of the carotenoids present in *S.platensis* [13].

The generation of biomass by photosynthetic *S.platensis* cultures depend on environmental factors including nitrogen concentration, light intensity, and pH [14-16]. Urea as a source of nitrogen is cheaper than nitrate [17]. The aim of this study were to assess the effect of nitrogen concentration, intensity of light, and pH of *S.platensis* in culture glasses.

MATERIALS AND METHODS

Test Chemical

S. platensis was obtained from BBPBAP (Balai Besar Pengembangan Budidaya Air Payau) Company, Indonesia in form living cell. Bold bassal medium, sodium bicarbonate, urea, aquadest, ascorbic acid, acetone, 1,1-diphenyl-2-picryl-hydrazyl free radicals, methanol, acetonitrile, dichloromethane.

Culture Condition

The S. Platensis cultures were maintained in 500 mL glass which is containing 300 mL bold bassal medium for each. Cultures was stirred by bubling air at room temperature and keep under fluorescent of 3000 lux with photoperiode dark : light (12:12 h/h) and sunlight for 19 days.

Productivity of biomass was calculated by measuring absorbance of cells. The absorbance of cell was determined by using spectrophotometer UV-vis (Genesys 20 Thermoscientific). The growth of the cell was cotrolled once in two days.

The optimum variation of this method was used for the next step. The following variations including the initial absorbance of cells were moderated by using spectrophotometer. The wavelength used was 560 nm. The pH of the cultures were measuredby pH meter and continued by adding $NaHCO_3$ gradually until gotten pH 8 and 9. Urea was used as a nitrogen source in this process.

Antioxidant activity

Free radical scavenging activity of different extract using acetone and acetone: methanol (1:1) from *S. platensis* was measured by DPPH method. In brief, 100 µL of DPPH 0.1 mM was added to 100 µL S.platensis extract into 48-wells microplate. The concentration of *S. platensis* was variated (500, 250, 125, dan 62.5 µg/mL). The mixture was incubated using room temperature for 30 min. The absorbance was measured at 517 nm by using spectrophotometer UV-vis (Shimadzu, Japan) [13]. The positive control used ascorbic acid and the experiment was done in triplicate [16].

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \text{ x } 100\ \%$$

HPLC Measurement

The homogenity and the purity of β-carotene were checked by HPLC (Shimadzu L17844, Japan) reverse phase 25 cm, C18 column with isocratic system consisted methanol: acetonitrile: dichloromethane (10:70:20, v/v/v) at flow rate 1.0 ml/ min and the column temperature was 25°C.The identification of β-carotene was performed by comparing retention time. β-carotene was monitored at 450 nm with a UV-visible detector (Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

The growth of *Spirulina platensis*

The growth of *S. platensis* cell in 300 mL culture using BBM standard under sunlight was showed in figure 1. The other growth curves were the same cultural cells among different condition (figure 2-5). The higher of absorbance of cells was selected for further sudies.

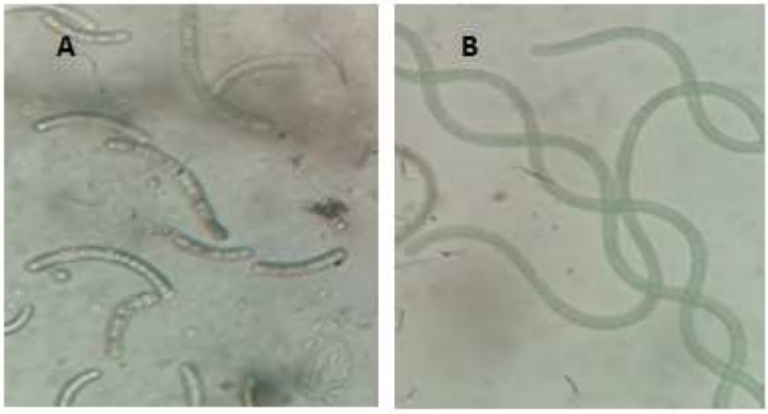

Figure 1. Morphology of *S.platensis* cells; *S.platensis* at second day (A). *S. platensis* at tenth day (B)

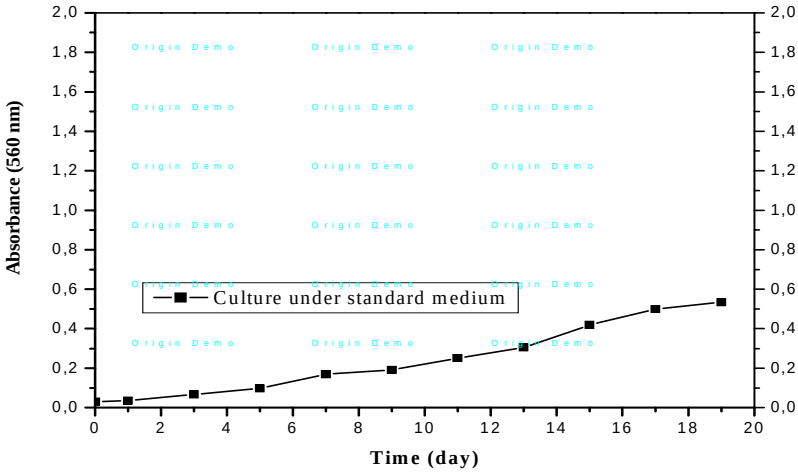

Figure 2. Shows the growth of *S. platensis* cell under sunlight using BBM standard

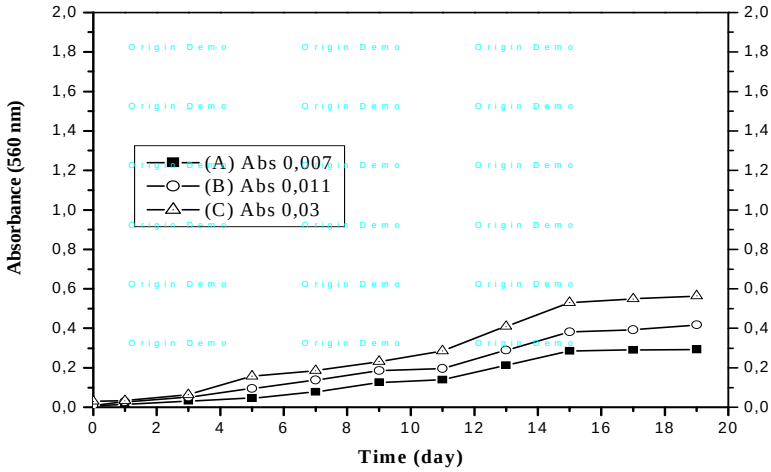

Figure 3. Effect of initial absorbance by using spectrophotometer 560 nm. *S. platensis* in culture glasses under natural sunlight. (A) initial absorbancewas 0.007; (B) initial absorbance was 0.011; (C) initial absorbance

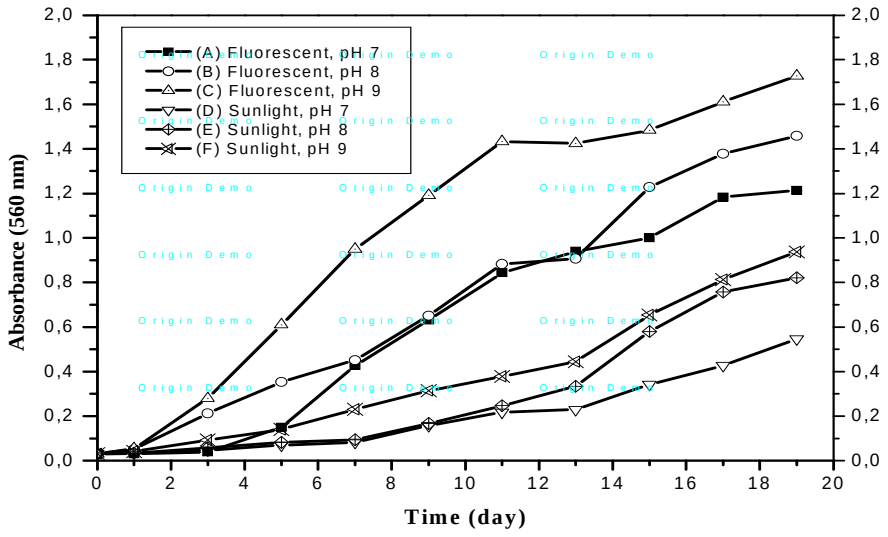

Figure 4. Effect of initial $NaHCO_3$ concentration on *S. platensis* cultivated in glass. (A-C) cultivated under fluorescent light (3000 lux); (A) culture when pH was 7; (B) culture when pH was 8; (C) culture when pH was 9. (D-F) cultivated under sunlight; (D) culture when pH was 7; (E) culture when pH was 8; (F) culture when pH was 9

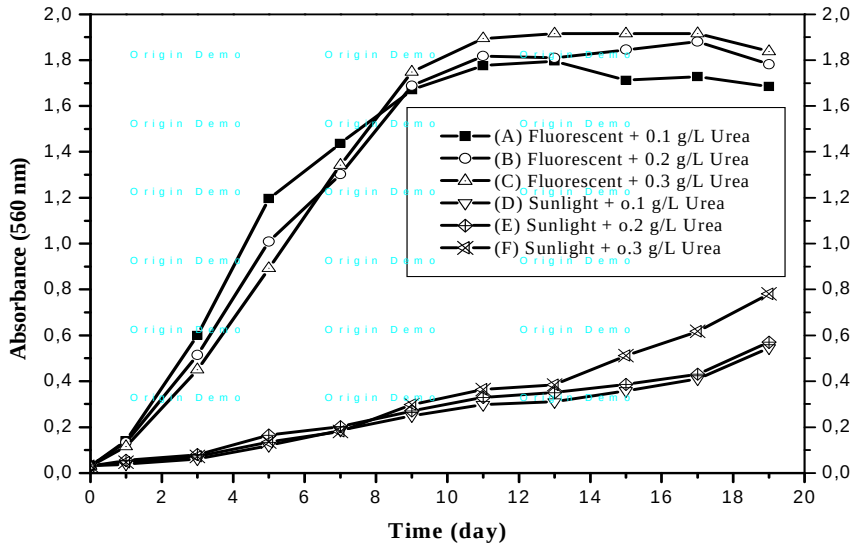

Figure 5. Effect of initial urea concentration on *S.platensis* cultivated in glass. (A-C) cultivated under fluorescent light (3000 lux); (A) culture using 0.1 g/L of urea; (B) culture using 0.2 g/L of urea (C) culture using 0.3 g/L of urea. (D-F) cultivated under sunlight; (D) culture using 0.1 g/L urea; (E) culture using 0.2 g/ of urea (F) culture using 0.3 g/L of urea

Culture of *S.platensis* was grown in modified medium various urea which was each containing nitrogen. After 9 days of cultivation at 3000 lux fluorescence light, the absorbance increased up to 1.7 and 0.25 under sunlight respectively (Fig. 5). Light intensity was an important factor to maximize the conversion of incident light energy to algae biomass. Zainul *et al* reported (2015) light intensity, in order to combination of, from indoors light (sunlight enter to the room) and illumination of room fluorescence light, which intensity region between 774-2000 lux(18), lower than 3000 lux fluorescence light.

Antioxidant Test

The *S. platensis* acetone: methanol (1:1) extract of this microalgae showed better antioxidant potential when compare to *S. platensis* acetone extract. Antioxidant acivities of various *S.platensis* extract were studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method as showed in figure 6.

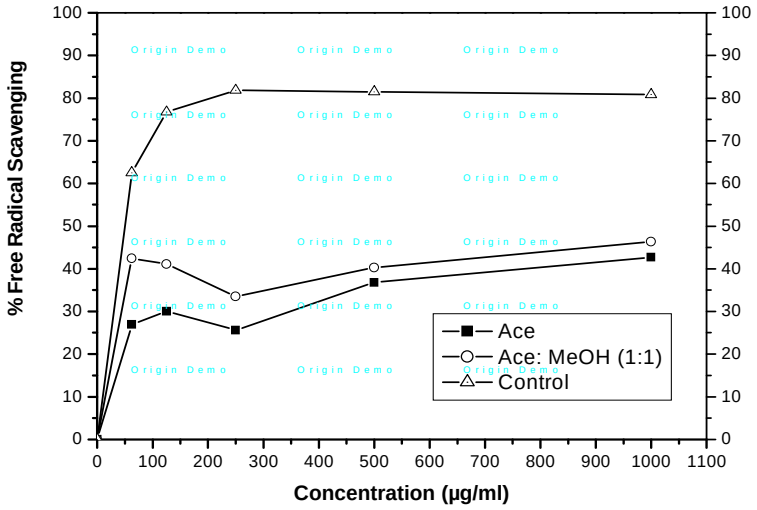

Figure 6. Effect of initial urea concentration on *S.platensis* and antioxidant acivities

HPLC analysis of β-carotene

The carotenoid compound especially β-carotene obtained from S. platensis produced several major peaks which were separated within 23.08 min (Figure 7.A) and 24.42 min (Figure 7.B) through a C18 column showed the available β-carotene. The detectable of β-carotene and other carotenoid undetectable were showed (Figure 7)

The purity calculated as the percentage of the β-carotene peak area was 2.58 and retention time was 23.08 whereas the acetone ectract displayed the highest sample area and produced 1.32% of β-carotene out of total biomass (Figure 7.A).

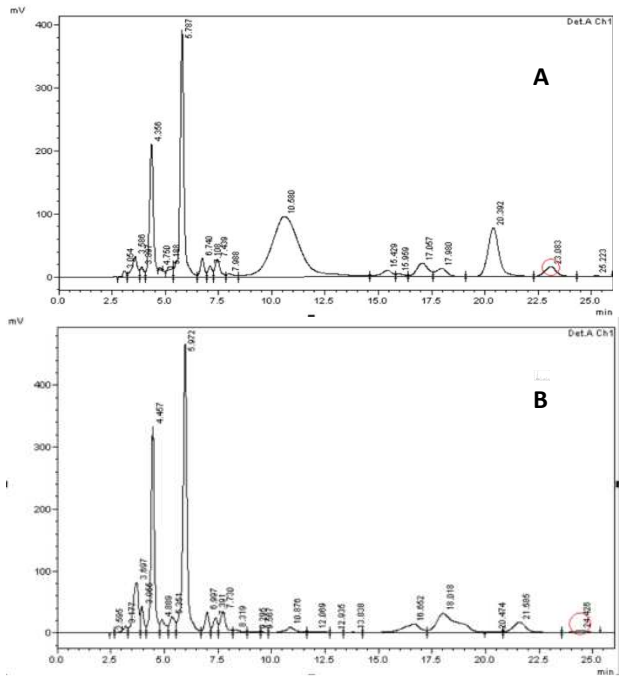

Figure 7. HPLC profile of total carotenoid extract from S. platensis extracted using acetone (Figure 7.A) S. platensis extracted using acetone: methanol (Figure 7.B)

CONCLUSION

The carotenoids are widely used as colorants, they also play an important role as a precursors of vitamin A and antioxidant. The optimum growth of *Spirulina platensis* was in 0.3 g/L urea, pH 9 at fluoresense light (3000 lux). Antioxidant acivity of various spirulina extract was studied by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging method. The Spirulina radicals scavenging activity of acetone:methanol (1:1) extract was 46.39 %. The

acetone:methanol (1:1) extract showed better antioxidant activity than acetone extract. The β-carotene was determined using HPLC in a silica gel column with dichloromethane, acetonitrile, methanol (20:70:10, v/v/v) as a mobile phase. The acetone extract displayed the highest sample area and produced 1.32% of β-carotene out of total biomass.

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105	Current Pharmaceutical Analysis	journal	ISSN 15734129	0.225	Q3	18	41	128
106	Dhaka University Journal of Pharmaceutical Scie	journal	ISSN 18161839, 18161820	0.225	Q3	8	16	87
107	International Journal of Drug Delivery Technolog	journal	ISSN 09754415	0.216	Q3	4	20	40

108	Pharmaceutical Engineering	journal	ISSN 02738139	0.215	Q3	11	53	180
109	Turkish Journal of Pharmaceutical Sciences	journal	ISSN 1304530X	0.215	Q3	8	22	117
110	American Pharmaceutical Review	journal	ISSN 10998012	0.212	Q3	17	66	218
111	Journal of Pharmaceutical Negative Results	journal	ISSN 09769234, 22297723	0.196	Q3	3	8	25
112	Clinical Medicine Insights: Therapeutics	journal	ISSN 1179559X	0.195	Q3	5	8	39
113	International Journal of Applied Pharmaceutics	journal	ISSN 09757058	0.194	Q3	5	8	28
114	International Journal of Pharmaceutical Science	journal	ISSN 0976044X	0.193	Q3	16	596	1483
115	International Journal of Drug Delivery	journal	ISSN 09750215	0.192	Q3	8	9	106
116	**Der Pharmacia Lettre**	**journal**	**ISSN 09755071**	**0.19**	**Q3**	**11**	**539**	**807**
117	Pharmaceutical Care Espana	journal	ISSN 11396202	0.189	Q3	8	30	107
118	Farmacevtski Vestnik	journal	ISSN 00148229	0.189	Q3	4	68	164
119	Pharmaceutical Care and Research	journal	ISSN 16712838	0.189	Q3	6	149	477
120	Korean Journal of Pharmacognosy	journal	ISSN 02533073	0.185	Q3	12	51	101
121	Journal of Chinese Pharmaceutical Sciences	journal	ISSN 10031057	0.184	Q3	6	108	265
122	Journal of Pharmaceutical Sciences and Resear	journal	ISSN 09751459	0.169	Q3	15	257	233
123	International Journal of Green Nanotechnology	journal	ISSN 19430906, 19430892	0.167	Q3	1	0	13
124	Pharmacy Education	journal	ISSN 14772701, 15602214	0.165	Q3	13	43	51
125	International Journal of Current Pharmaceutical	journal	ISSN 0976822X	0.165	Q3	5	43	37
126	Acta Farmaceutica Bonaerense	journal	ISSN 03262383	0.162	Q3	18	271	765
127	International Journal of Research in Ayurveda ar	journal	ISSN 22774343, 22293566	0.162	Q3	4	3	512
128	Journal of Generic Medicines	journal	ISSN 17411343	0.16	Q3	11	12	85
129	Jordan Journal of Pharmaceutical Sciences	journal	ISSN 19957157	0.157	Q3	6	18	63
130	Ceska a Slovenska Farmacie	journal	ISSN 12107816	0.155	Q3	13	20	122
131	Open Vaccine Journal	journal	ISSN 18750354	0.154	Q3	7	0	6
132	Farmatsija	journal	ISSN 04280296	0.149	Q3	4	16	51
133	Journal of Bioequivalence and Bioavailability	journal	ISSN 09750851	0.147	Q3	6	0	120
134	Pharmaceutical Technology	journal	ISSN 15432521	0.145	Q3	30	140	532
135	Open Pharmacology Journal	journal	ISSN 18741436	0.144	Q3	4	0	9
136	Chinese Pharmaceutical Journal	journal	ISSN 10012494	0.143	Q3	17	417	1420
137	International Journal of Pharmaceutical Science	journal	ISSN 09754725	0.14	Q3	6	0	66
138	Current Trends in Biotechnology and Pharmacy	journal	ISSN 09738916	0.138	Q3	8	33	131
139	Systematic Reviews in Pharmacy	journal	ISSN 09758453	0.136	Q3	7	1	14
140	International Journal of Pharmaceutical Compou	journal	ISSN 10924221	0.135	Q3	6	48	229
141	Journal of Bioanalysis and Biomedicine	journal	ISSN 1948593X	0.135	Q3	6	0	66
142	Journal of Chemical and Pharmaceutical Resear	journal	ISSN 09757384	0.135	Q3	21	2	3533
143	Revista de Ciencias Farmaceuticas Basica e Ap	journal	ISSN 18084532	0.131	Q3	10	1	238
144	Chinese Traditional and Herbal Drugs	journal	ISSN 02532670	0.13	Q3	10	565	1836

145	International Journal of Green Nanotechnology:	journal	ISSN 1943085X, 19430906	0.13	Q3	7	0	64
146	Indian Drugs	journal	ISSN 0019462X	0.123	Q3	29	0	147
147	Journal of Pharmacy Technology	journal	ISSN 87551225	0.123	Q3	8	42	116
148	Journal of Chemical and Pharmaceutical Scienc	journal	ISSN 09742115	0.123	Q3	3	520	241
149	European Journal of Parenteral and Pharmaceu	journal	ISSN 17406277, 09644679	0.121	Q4	3	0	68
150	Pharmazeutische Industrie	journal	ISSN 0031711X, 16167074	0.12	Q4	16	267	726
151	Journal of China Pharmaceutical University	journal	ISSN 10005048	0.12	Q4	12	101	307
152	Revista Cubana de Farmacia	journal	ISSN 00347515, 15612988	0.119	Q4	5	64	168
153	BioPharm International	journal	ISSN 1542166X	0.118	Q4	21	96	370
154	SA Pharmaceutical Journal	journal	ISSN 10151362	0.116	Q4	3	100	348
155	Klinicka Farmakologie a Farmacie	journal	ISSN 12127973, 18035353	0.115	Q4	3	38	114
156	International Journal of Pharmaceutical and Clin	journal	ISSN 09751556	0.115	Q4	4	60	108
157	Bulletin of Pharmaceutical Sciences	journal	ISSN 11100052	0.115	Q4	4	0	18
158	Farmaceutski Glasnik	journal	ISSN 00148202	0.113	Q4	5	56	227
159	Ars Pharmaceutica	journal	ISSN 00042927	0.112	Q4	14	25	93
160	Actualites Pharmaceutiques Hospitalieres	journal	ISSN 17697344	0.112	Q4	2	0	3
161	Thai Journal of Pharmaceutical Sciences	journal	ISSN 01254685	0.112	Q4	8	21	194
162	Orphan Drugs: Research and Reviews	journal	ISSN 22306161	0.112	Q4	1	10	2
163	Anales de la Real Academia Nacional de Farma	journal	ISSN 16974271, 1697428X	0.111	Q4	7	39	102
164	Pharmakeftiki	journal	ISSN 11054999	0.111	Q4	2	8	39
165	Eurasian Journal of Analytical Chemistry	journal	ISSN 13063057	0.111	Q4	3	15	38
166	U.S. Pharmacist	journal	ISSN 01484818	0.11	Q4	5	181	459
167	Journal of International Pharmaceutical Researc	journal	ISSN 16740440	0.11	Q4	6	152	447
168	Yakugaku Zasshi	journal	ISSN 00316903	0.109	Q4	32	188	375
169	Iranian Journal of Pharmaceutical Sciences	journal	ISSN 17352444	0.109	Q4	5	0	83
170	Pharmaceutical Journal	journal	ISSN 00316873	0.107	Q4	24	347	1135
171	Journal de Pharmacie de Belgique	journal	ISSN 00472166	0.107	Q4	11	21	95
172	Acta Pharmaceutica Hungarica	journal	ISSN 00016659	0.106	Q4	10	13	52
173	Open Bioactive Compounds Journal	journal	ISSN 18748473	0.106	Q4	5	0	4
174	Pharmaceutical Outsourcing	journal	ISSN 19453337, 19453345	0.106	Q4	4	15	119
175	Clinical Research and Regulatory Affairs	journal	ISSN 10601333, 15322521	0.105	Q4	12	15	24
176	Revista Mexicana de Ciencias Farmaceuticas	journal	ISSN 10273956	0.105	Q4	4	0	68
177	Ankara Universitesi Eczacilik Fakultesi Dergisi	journal	ISSN 10153918	0.104	Q4	6	0	6
178	Farmaceutico Hospitales	journal	ISSN 02144697	0.104	Q4	3	0	10
179	EBR - European Biopharmaceutical Review	journal	ISSN 13690663	0.103	Q4	3	0	165
180	Journal of Global Pharma Technology	journal	ISSN 09758542	0.103	Q4	7	0	52
181	Manufacturing Chemist	journal	ISSN 02624230	0.102	Q4	5	16	364

182	Pharmazie in Unserer Zeit	journal	ISSN 00483664, 16151003	0.102	Q4	9	0	78
183	Journal of Pharmacy of Istanbul University	journal	ISSN 03677524	0.102	Q4	4	0	10
184	Pharmaceutical biotechnology	journal	ISSN 10780467, 10058915	0.101	Q4	21	0	310
185	Anales de la Real Academia Nacional de Medici	journal	ISSN 00340634	0.101	Q4	6	0	36
186	Pharmaceutical Technology Europe	journal	ISSN 01646826	0.101	Q4	14	146	284
187	Fabad Journal of Pharmaceutical Sciences	journal	ISSN 13004182	0.101	Q4	10	0	23
188	PZ Prisma	journal	ISSN 09455566	0.1	Q4	5	30	105
189	Drug Topics	journal	ISSN 00126616	0.1	Q4	7	288	742
190	EPC - European Pharmaceutical Contractor	journal	ISSN 1364369X	0.1	Q4	2	19	176
191	Farmaceuticky Obzor	journal	ISSN 00148172	0.1	Q4	6	0	77
192	S.T.P. Pharma Pratiques	journal	ISSN 11571497	0.1	Q4	10	0	104
193	Arhiv za Farmaciju	journal	ISSN 00041963	0.1	Q4	2	0	107
194	Drug Delivery System	journal	ISSN 09135006	0.1	Q4	2	53	90

Total Refs.	Total Cites	Citable Do	Cites / Doc	Ref. / Doc.	Country
5748	1986	125	13.53	78.74	Netherlands
24836	7585	436	15.97	118.83	Netherlands
0	17	1	17	0	Egypt
35098	11504	1348	7.66	60.62	Netherlands
17416	2916	480	5.79	61.76	United Kingdo
10198	3166	478	6.08	48.79	United States
14408	3294	713	4.6	49.68	United States
17838	5754	1191	4.73	46.94	United States
2405	839	270	3.29	41.47	United States
7783	1426	385	3.61	58.08	Netherlands
12285	1914	377	5.02	91	United Kingdo
14015	4309	1049	3.7	34.18	United States
17478	3292	751	4.13	54.62	Netherlands
36482	9501	2254	4.18	39.31	Netherlands
3823	752	148	4.45	36.41	United Kingdo
9874	2561	761	3.24	42.74	United States
3690	738	203	4.5	83.86	Switzerland
1527	217	42	5.2	218.14	United States
6354	1430	360	3.56	45.39	United States
12508	3082	830	3.61	43.43	United States
193	27	5	5.4	64.33	New Zealand
10982	3040	763	3.93	40.08	Netherlands
1700	421	144	2.75	35.42	United States
31152	6728	2256	2.94	35.89	United Kingdo
15548	4394	1423	3.14	30.37	Netherlands
4891	1990	601	3.09	29.82	Germany
19646	3574	1208	2.82	32	United States
10050	1552	639	2.39	38.36	United Kingdo
9627	2303	602	3.71	55.33	United States
30457	9857	3902	2.56	25.92	United Kingdo
23389	1317	423	3.03	44.55	New Zealand
3906	1002	410	2.65	20.45	United Kingdo
2477	519	203	1.88	35.9	Japan

8390	1168	484	1.9	63.56	Netherlands
7958	811	331	2.18	64.18	United Kingdo
5790	746	264	2.7	60.95	United Kingdo
1205	197	80	2.81	38.87	Iran
3299	173	87	1.99	48.51	United States
4351	983	474	1.83	19.87	United States
1350	355	145	2.4	20.77	United Kingdo
1795	272	105	2.77	48.51	Switzerland
2800	278	135	2.13	53.85	United States
8714	1371	645	1.95	41.5	Germany
57	70	30	1.74	19	United States
6409	1288	538	2.29	31.11	United Kingdo
2315	334	139	2.33	47.24	Canada
5249	320	152	1.94	30.88	United Kingdo
8846	1677	932	1.68	33.38	Japan
3538	653	292	2.14	31.59	Germany
3505	377	203	1.87	26.96	United States
1679	576	326	1.82	29.46	Germany
2014	201	139	1.32	36.62	United Kingdo
992	219	110	1.62	26.11	United Kingdo
3531	631	427	1.42	25.59	Netherlands
1745	110	58	1.45	72.71	Netherlands
2918	389	143	2.18	33.93	United States
3072	408	177	2.45	19.95	Netherlands
9100	990	623	1.63	34.73	United Kingdo
3286	368	232	1.57	34.23	United Kingdo
945	105	87	1.39	37.8	United States
3935	464	260	1.59	44.21	United Kingdo
2721	492	359	1.46	22.12	United States
4298	367	209	1.45	51.78	Netherlands
953	218	120	1.45	23.83	Croatia
833	53	99	0.69	24.5	United Kingdo
1527	175	117	1.89	26.79	China
0	2	1	2	0	Netherlands
3372	654	513	1.2	27.64	Germany
1439	201	165	1.01	16.35	United Kingdo
761	133	85	1.26	19.03	India

943	116	99	1.07	28.58	Spain
1304	135	128	1.16	19.46	United States
34	35	22	0.78	34	Netherlands
2192	253	235	0.9	28.1	India
0	25	21	1.4	0	United States
0	116	82	1.56	0	United States
2940	317	301	1.01	24.5	India
0	80	82	1.02	0	United Kingdo
1005	268	207	1.08	25.13	Austria
2752	537	519	1.03	14.19	United Kingdo
1127	29	40	0.76	26.83	Netherlands
224	35	39	0.86	20.36	United States
3479	369	391	0.98	34.11	Netherlands
4390	420	433	0.85	29.86	Poland
2108	148	145	1.2	32.94	Netherlands
12216	740	1221	0.48	26.97	India
6751	506	628	0.73	20.21	Pakistan
713	65	69	0.9	39.61	Netherlands
517	53	70	0.45	16.16	United States
6500	452	666	0.66	30.09	United States
2024	156	167	0.91	38.92	United States
116	21	14	1.7	38.67	Netherlands
4665	404	389	0.98	44.86	Netherlands
27837	1963	3729	0.49	29.65	India
0	86	147	0.51	0	United States
827	86	127	0.78	13.56	Canada
7007	383	572	0.62	22.75	Nigeria
5004	180	248	0.75	43.14	France
0	247	313	0.79	0	India
2025	86	131	0.66	32.14	France
599	33	47	0.5	35.24	China
10850	335	745	0.37	29.73	India
908	69	161	0.4	30.27	India
0	14	21	0	0	Netherlands
1380	84	125	0.9	33.66	Netherlands
301	19	86	0.14	18.81	Bangladesh
509	17	40	0.33	25.45	India

515	32	156	0.17	9.72	United States
746	47	114	0.21	33.91	Turkey
782	67	203	0.28	11.85	United States
141	12	25	0.48	17.63	India
684	17	39	0.65	85.5	New Zealand
202	15	28	0.67	25.25	India
16708	454	1483	0.32	28.03	India
207	43	106	0.44	23	United States
13602	**354**	**807**	**0.44**	**25.24**	**India**
459	8	85	0.1	15.3	Spain
1397	8	152	0.08	20.54	Slovenia
1329	37	476	0.07	8.92	China
1396	38	101	0.38	27.37	South Korea
2745	97	260	0.35	25.42	China
5712	76	233	0.28	22.23	India
0	5	11	0.5	0	United Kingdo
945	16	51	0.29	21.98	Netherlands
1384	11	37	0.24	32.19	India
6158	232	757	0.31	22.72	Argentina
107	85	512	0.18	35.67	India
217	14	74	0.14	18.08	United Kingdo
505	13	63	0.17	28.06	Jordan
511	26	115	0.24	25.55	Czech Republ
0	3	5	0	0	Netherlands
627	8	51	0.17	39.19	Bulgaria
0	46	88	0.56	0	United States
611	41	499	0.07	4.36	United States
0	3	8	0	0	Netherlands
7277	247	1420	0.2	17.45	China
0	16	66	0	0	India
968	25	131	0.15	29.33	India
31	3	14	0.29	31	India
0	55	223	0.24	0	United States
0	16	44	0.27	0	United States
207	943	3532	0.2	103.5	India
24	45	238	0.16	24	Brazil
4845	273	1663	0.14	8.58	China

0	25	63	0	0	United States
0	19	147	0.08	0	India
908	16	113	0.14	21.62	United States
6617	31	241	0.11	12.73	India
0	6	56	0.11	0	United Kingdo
1997	16	705	0.03	7.48	Germany
2397	50	307	0.16	23.73	China
1533	8	157	0.05	23.95	Cuba
418	35	347	0.07	4.35	United States
795	18	223	0.05	7.95	South Africa
672	6	99	0.06	17.68	Czech Republ
1596	19	108	0.16	26.6	Australia
0	3	17	0	0	Egypt
296	4	186	0.02	5.29	Croatia
777	5	90	0.03	31.08	Spain
0	0	2	0	0	Netherlands
513	16	192	0.13	24.43	Thailand
924	0	2	0	92.4	New Zealand
1536	3	84	0.03	39.38	Spain
509	1	34	0	63.63	Greece
357	7	38	0.15	23.8	Turkey
3006	24	375	0.07	16.61	United States
3912	34	445	0.09	25.74	China
3959	60	309	0.19	21.06	Japan
0	10	82	0.14	0	Iran
787	15	466	0.03	2.27	United Kingdo
0	15	85	0.29	0	Belgium
276	6	52	0.11	21.23	Hungary
0	1	4	0.25	0	Netherlands
123	4	101	0.04	8.2	United States
767	9	20	0.42	51.13	United States
0	4	60	0.1	0	Mexico
0	0	6	0	0	Turkey
0	0	6	0	0	Spain
0	2	158	0.02	0	United Kingdo
0	1	52	0	0	India
51	1	324	0	3.19	United Kingdo

0	4	66	0	0	Germany
0	0	10	0	0	Turkey
0	9	310	0.04	0	United States
0	0	36	0	0	Spain
747	1	221	0.01	5.12	United States
0	1	23	0	0	Turkey
492	0	93	0	16.4	Germany
79	2	75	0.03	0.27	United States
75	0	162	0	3.95	United Kingdo
0	1	67	0.03	0	Slovakia
0	0	101	0	0	France
0	0	106	0	0	Serbia
622	0	63	0	11.74	Japan

PERINGKAT JURNAL DER PHARMACIA LETTRE DI ASIA

Rank	Title	Type	Issn	SJR	JR Quarti	H index	l Docs. (2
1	Drug metabolism and pharmacokinetics	journal	ISSN 13474367	0.78	Q1	49	69
2	Biological and Pharmaceutical Bulletin	journal	ISSN 09186158, 13475	0.644	Q1	88	265
3	Asian Journal of Pharmaceutical Sciences	journal	ISSN 18180876	0.464	Q2	11	57
4	Journal of Advanced Pharmaceutical Technolog	journal	ISSN 09762094, 01105	0.427	Q2	15	40
5	Pharmacognosy Magazine	journal	ISSN 09764062, 09731	0.376	Q2	19	78
6	Indian Journal of Pharmaceutical Sciences	journal	ISSN 19983743, 02504	0.371	Q2	40	120
7	Asian Journal of Pharmaceutical and Clinical Re	journal	ISSN 09742441	0.326	Q2	16	453
8	Pakistan Journal of Pharmaceutical Sciences	journal	ISSN 1011601X	0.316	Q2	28	334
9	International Journal of Pharmacy and Pharmac	journal	ISSN 09751491	0.282	Q2	24	939
10	Journal of Pharmacy Research	journal	ISSN 09746943, 00974	0.253	Q2	8	0
11	Journal of Exercise Science and Fitness	journal	ISSN 1728869X	0.238	Q3	14	17
12	International Journal of PharmTech Research	journal	ISSN 09744304	0.23	Q3	28	365
13	International Journal of Green Pharmacy	journal	ISSN 19984103, 09738	0.229	Q3	13	30
14	Dhaka University Journal of Pharmaceutical Sci	journal	ISSN 18161839, 18161	0.225	Q3	8	16
15	International Journal of Drug Delivery Technolo	journal	ISSN 09754415	0.216	Q3	4	20
16	Journal of Pharmaceutical Negative Results	journal	ISSN 09769234, 22297	0.196	Q3	3	8
17	International Journal of Applied Pharmaceutics	journal	ISSN 09757058	0.194	Q3	5	8
18	International Journal of Pharmaceutical Science	journal	ISSN 0976044X	0.193	Q3	16	596
19	***Der Pharmacia Lettre***	***journal***	***ISSN 09755071***	***0.19***	***Q3***	***11***	***539***
20	Pharmaceutical Care and Research	journal	ISSN 16712838	0.189	Q3	6	149
21	Korean Journal of Pharmacognosy	journal	ISSN 02533073	0.185	Q3	12	51
22	Journal of Chinese Pharmaceutical Sciences	journal	ISSN 10031057	0.184	Q3	6	108
23	Journal of Pharmaceutical Sciences and Resea	journal	ISSN 09751459	0.169	Q3	15	257
24	International Journal of Current Pharmaceutical	journal	ISSN 0976822X	0.165	Q3	5	43
25	International Journal of Research in Ayurveda a	journal	ISSN 22774343, 22293	0.162	Q3	4	3
26	Chinese Pharmaceutical Journal	journal	ISSN 10012494	0.143	Q3	17	417
27	International Journal of Pharmaceutical Science	journal	ISSN 09754725	0.14	Q3	6	0
28	Current Trends in Biotechnology and Pharmacy	journal	ISSN 09738916	0.138	Q3	8	33
29	Systematic Reviews in Pharmacy	journal	ISSN 09758453	0.136	Q3	7	1
30	Journal of Chemical and Pharmaceutical Resea	journal	ISSN 09757384	0.135	Q3	21	2
31	Chinese Traditional and Herbal Drugs	journal	ISSN 02532670	0.13	Q3	10	565
32	Indian Drugs	journal	ISSN 0019462X	0.123	Q3	29	0
33	Journal of Chemical and Pharmaceutical Scienc	journal	ISSN 09742115	0.123	Q3	3	520

34	Journal of China Pharmaceutical University	journal	ISSN 10005048	0.12	Q4	12	101
35	Thai Journal of Pharmaceutical Sciences	journal	ISSN 01254685	0.112	Q4	8	21
36	Journal of International Pharmaceutical Researc	journal	ISSN 16740440	0.11	Q4	6	152
37	Yakugaku Zasshi	journal	ISSN 00316903	0.109	Q4	32	188
38	Journal of Global Pharma Technology	journal	ISSN 09758542	0.103	Q4	7	0
39	Drug Delivery System	journal	ISSN 09135006	0.1	Q4	2	53

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Docs. (3y	Total Refs	Cites (3ye	Docs. (3	/ Doc. (2y	Ref. / Doc	Country
239	2477	519	203	1.88	35.9	Japan
967	8846	1677	932	1.68	33.38	Japan
117	1527	175	117	1.89	26.79	China
104	761	133	85	1.26	19.03	India
241	2192	253	235	0.9	28.1	India
305	2940	317	301	1.01	24.5	India
1222	12216	740	1221	0.48	26.97	India
628	6751	506	628	0.73	20.21	Pakistan
3740	27837	1963	3729	0.49	29.65	India
313	0	247	313	0.79	0	India
47	599	33	47	0.5	35.24	China
745	10850	335	745	0.37	29.73	India
163	908	69	161	0.4	30.27	India
87	301	19	86	0.14	18.81	Bangladesh
40	509	17	40	0.33	25.45	India
25	141	12	25	0.48	17.63	India
28	202	15	28	0.67	25.25	India
1483	16708	454	1483	0.32	28.03	India
807	***13602***	***354***	***807***	***0.44***	***25.24***	***India***
477	1329	37	476	0.07	8.92	China
101	1396	38	101	0.38	27.37	South Korea
265	2745	97	260	0.35	25.42	China
233	5712	76	233	0.28	22.23	India
37	1384	11	37	0.24	32.19	India
512	107	85	512	0.18	35.67	India
1420	7277	247	1420	0.2	17.45	China
66	0	16	66	0	0	India
131	968	25	131	0.15	29.33	India
14	31	3	14	0.29	31	India
3533	207	943	3532	0.2	103.5	India
1836	4845	273	1663	0.14	8.58	China
147	0	19	147	0.08	0	India
241	6617	31	241	0.11	12.73	India

307	2397	50	307	0.16	23.73	China
194	513	16	192	0.13	24.43	Thailand
447	3912	34	445	0.09	25.74	China
375	3959	60	309	0.19	21.06	Japan
52	0	1	52	0	0	India
90	622	0	63	0	11.74	Japan

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