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## Isolation and Identification of Freshwater Microalgae Potentially as Antibacterial From Talago Biru, Koto Baru, West Sumatera

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### ABSTRACT

Microalgae have potential as antibacterial compounds, the content of microalgae which has the activity of amino acids, terpenoids, florotanins, steroids, phenolics, acrylic acids and halogenated ketones can be used as an antimicrobial. In this research, screening and isolation of microalgae which can be used as antibacterial has been done at Talago Biru, Koto Baru, West Sumatera and the content of secondary metabolites were identified. The isolation was done by using agar plate and serial dilution methods. Isolates were identified morphological and molecular, it is known as a type of *Chlorella* sp. Best harvest time was determined by Spectrophotometric method at 450 nm is obtained in day 20 on the stationary phase. Based on phytochemical test, this isolate positively contain phenolics, saponins, steroids and triterpenoids, then the antibacterial activity of microalgae extracted with methanol was tested. It is known that the isolates has potential as an antibacterial, were seen from the test results provided by the zone of inhibition against four isolates of bacteria test with extract concentrations of 10 mg / mL, 100 mg / mL and 500 mg / mL respectively *E. Coli* 8 mm, 10 mm and 12 mm, *Salmonella bacteria typhii* 10.5 mm, 9 mm, 9 mm, *Staphylococcus aureus* 9 mm, 10 mm and 10 mm as well as the bacteria *Bacillus cereus* 10 mm, 10.5 mm, and 11 mm with a comparator amoxycillin as a positive control and 0.1% DMSO as a negative control.

**Keywords:** Talago Biru, *Chlorella* sp., secondary metabolite, antibacterial

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### INTRODUCTION

Microalgae have been used in the production of biomass, energy production, bioaccumulation of specific compounds as well as a variety of biotransformation processes. The products produced by microalgae is intracellular and extracellular, ranging from simple metabolites to antibiotic complex, toxin pigments as well as a number of other useful products<sup>[1]</sup>. Has many biological active substance that has been extracted from microalgae such as antialga, antiviral activity, and antimicrobial activity such as antibacterial and antifungal.<sup>[2]</sup>

Antimicrobials are biologically active molecules which are increasingly being used for animal and human health to prevent or treat infections caused by microbes<sup>[3]</sup>. The content of microalgae which has the activity of amino acids, terpenoids, florotanin, steroids, phenolic, acrylic acid and halogenated ketones can be used as an antimicrobial and anticancer<sup>[4]</sup>. *Chlorella vulgaris* and *Chlamydomonas pyrenoidosa* has been shown to have antibacterial activity in vitro against gram positive and negative<sup>[5]</sup>.

Other microalgae that has been investigated as antibacterial are *Pithophora oedogonium* extracted using ethanol can inhibit the growth of bacteria *Salmonella* and *Staphylococcus* sp<sup>[6]</sup>. *Sargassum wightii*, *Chaetomorpha linum*, *Padina Gymnospora*. with methanol and acetone extracts can inhibit the growth of bacteria *P. aeruginosa*, *S. typhi*-B, *Erwinia amylovora*, *Proteus vulgaris*, *E. coli* and *S. aureus*<sup>[7]</sup>. *Chlorella* sp. with ethanol extract can inhibit the growth of bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Yersinia enterocolitica*<sup>[8]</sup>. In this research was conducted to obtain a superior kind of freshwater microalgae derived from Talago Biru, Koto Baru, West Sumatera and observe the antimicrobial capabilities possessed by microalgae which have been isolated.

## MATERIALS AND METHODS

### Materials and Equipment

The materials were used in this study are water samples of microalgae from Talago Biru, Koto Baru, Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*), Medium BBM (NaNO<sub>3</sub>(Merck) , MgSO<sub>4</sub>.7H<sub>2</sub>O (Merck), NaCl(Merck), K<sub>2</sub>HPO<sub>4</sub> (Merck), KH<sub>2</sub>PO<sub>4</sub> (Merck), CaCl<sub>2</sub>.2H<sub>2</sub>O (Merck), ZnSO<sub>4</sub>.7H<sub>2</sub>O (Merck), MnCl<sub>2</sub>.4H<sub>2</sub>O (Merck), MoO<sub>3</sub> (Merck), CoSO<sub>4</sub> .5H<sub>2</sub>O (Merck), Co(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O (Merck), Qiagen kits for the isolation of DNA, loading dye, DMSO, H<sub>2</sub>SO<sub>4</sub>, technical methanol, Dragendorff reagents, meyer reagents, chloroform, ethanol 70%, FeCl<sub>3</sub> 5%, magnesium powder, 37% HCl, 1 N NaOH, Mueller-Hinton agar, Nutrient Agar, discs, amoxycillin.

The tools were used are 30 micron filter plankton net, a light microscope (Olympus CX41), Incubators, aerator, autoclave, UV-Vis spectrophotometer (Genesys 20), PCR tools (Polymerase Chain Reaction), electrophoresis apparatus, centrifuges, low temperature refrigerator, micropipette, Eppendorf tubes, vortex.

### Research procedure

#### Sampling Microalgae

Samples microalgae were collected from three withdrawal points with a depth of ± 1 meter from the surface of the water collected into one from Talago Biru, Koto Baru using a plankton net with 30 micron hole size.<sup>[9]</sup>

#### Isolation and Purification of Microalgae

*Bold Basal Medium*(BBM) was autoclaved at a pressure of 1.2 atm, temperature 121°C. 10 mL water sample was inoculated into 200 ml of medium in a 500-ml bottle, and then incubated with aeration administration. Every two days, the samples that had been grown were checked to see the growth of microalgae using a light microscope, and the dilution series had been done to see the growth.<sup>[10]</sup>

#### Morphological Identification of Microalgae

Morphological identification of microalgae which has been isolated is done microscopically. Observations were carried out regularly under the microscope to make sure that had gotten a single cell.

**Observation of Microalgae Growth**

Isolated microalgae growth is determined by Optical Density (OD) and measured using a spectrophotometer at a wavelength of 450 nm.<sup>[10]</sup>

**Molecular Identification**

Cells were harvested from 10 mL of liquid culture and DNA was isolated using a Qiagen DNA isolation kit. DNA isolation results were amplified using PCR amplification with 2 mL of genomic DNA, 0.2 mM deoxynucleotide trifospat, 1.25 units of Taq DNA Polymerase and use forward primer ChloroF 5'-CCT TGG TGT ATC TTG TTG GTC-3' and reverse primer ChloroR 5'-GAA TCA ACC TGA CAA GGC AAC-3'. For CHLORO amplification, program PCR consisted of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 59°C for 1 minute, and 72°C for 1 minute, with the cycles of the 10-minute extra at 72°C. DNA amplification product was electrophoresed with 1% agarose gel with a voltage of 101 V using 1x TAE buffer was detected by staining using loading dye. PCR products were purified with the addition Geneaid Gel / PCR DNA Fragments Extraction Kit<sup>[11]</sup> and sequencing was done in Macrogen, Korea.

**Phytochemical analysis**

Phytochemical analysis was conducted on the test alkaloids, triterpenoids and steroids, phenols, flavonoids and saponins. The analytical method used is based on Harborne (1987).

**Biomass collection and Making Microalgae Extract**

Liquid cultures of microalgae are dried on a predetermined harvesting, dry by the wind until all the water is gone. Dry biomass was added by ten parts technical methanol, then sonicated for 45 minutes. The filtrate obtained is filtered, the filtering is done several times until methanol is relatively colorless. Methanol was evaporated to obtain a dry extract of microalgae.

**Making suspension Bacteria**

Test bacterias (*E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*) were cultured on a nutrient agar medium for 24 hours at 37°C, then taken a loopful and suspended into 5 mL LB medium.<sup>[12]</sup>

**Testing the antibacterial activity**

Sterilized Mueller-Hinton agar liquid put into petri dishes 20 mL per each and allowed to solidify at room temperature. Media drip with 200 µL test bacterial suspension. Sterile paper disc with a diameter of 5 mm was dropped extract with a volume of 20 mL with a concentration of 10 mg / mL, 100 mg / mL and 500 mg / mL, and then placed on medium and incubated at room temperature for 24 hours. *Amoxycillin* 10 mg/mL was used as positive control and a negative control using DMSO 0.1%. Diameter of inhibition zone measured horizontally and vertically using a scale ruler.<sup>[12]</sup>

**RESULTS AND DISCUSSION****Microalgae Morphology and Molecular Identification Results Isolation**

Microalgae isolated from Talago Biru, Koto Baru, West Sumatera and obtained a single dominant isolates live in medium BBM. Isolates were identified morphological and molecular. Results of morphological identification of single isolates are then compared with the morphological data contained on the website Algalbase.org data which gathering microalgae that have been studied worldwide in Figure 1.

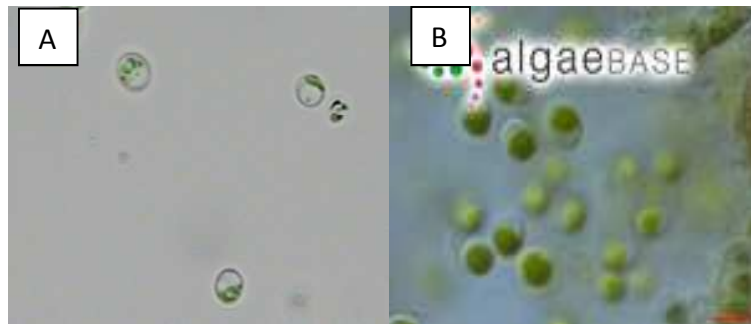


Figure 1. (A) Morphology of the isolated microalgae 1000x magnification, (B) Morphology *Chlorella sp.* of Algaebase.org

Judging from their morphological similarity, it can be said that the superior microalgae have been successfully isolated is a type of microalgae *Chlorella sp.* *Chlorella sp.* is a kind of green microalgae, In Figure 1 (A) it can be seen that the color of isolates is green. The green color signifies *Chlorella sp.* have chlorophyll. *Chlorella sp.* have a size of 3-15 microns and round, a eukaryotic organism which means that already have a cell nucleus and also *Chlorella sp.* does not have flagell<sup>[13]</sup>. From the result of morphological test we can state that single isolates of microalgae which has been isolated is the type *Chlorella sp.*, The results of morphological identification is confirmed by the results of existing molecular identification. Where the results of the identification of molecular phylogenetic trees obtained stating closest kinship microalgae isolation results can be seen in Figure 2.

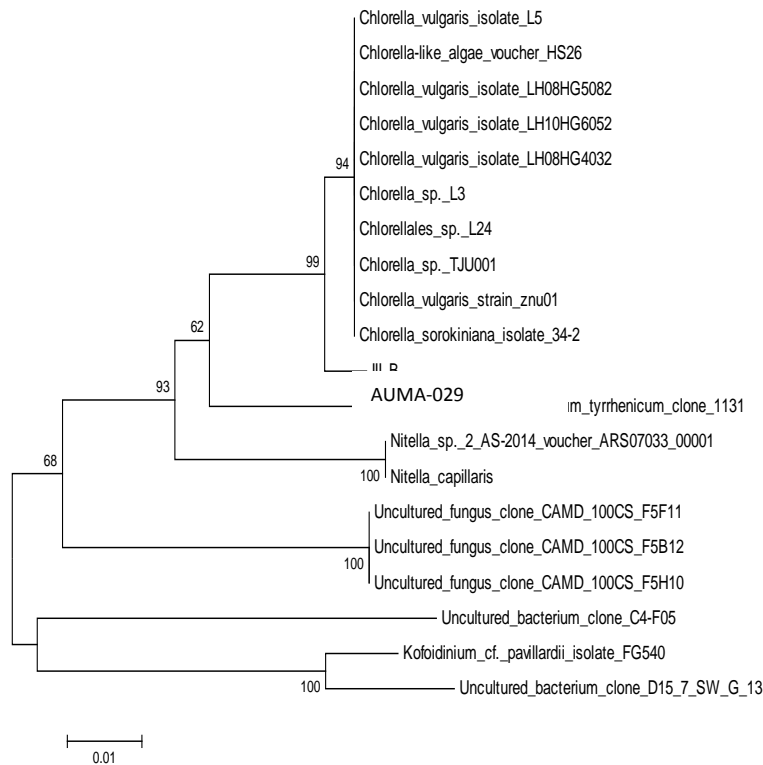


Figure 2. Results of analysis of phylogenetic trees with MEGA5.1

Phylogenetic trees obtained from it is seen that isolates AUMA-029 has the closest kinship with *Chlorella sp.* where the similarity percentage that obtained from Figure 2 is equal to 99%. The percentage of similarity is so great that it can be said that isolates AUMA-029 which was isolated from Talago Biru, Koto Baru is a type of *Chlorella sp.*

If the results of the identification of this molecular was compared with the result of morphological test it is proved that the isolates AUMA-029 is a group of *Chlorella sp.* In the morphological test was seen many similarities between isolates AUMA-029 with microalgae *Chlorella sp.* morphology which contained in the Algaebasedata. The more the similar characteristic, then the closer kinship.<sup>[15]</sup>

### Microalgae Growth Curve

Growth is defined as an increase in cell mass and size accompanied by the synthesis of macromolecules that generate new structures<sup>[15]</sup>. Microalgae growth curve can be seen in Figure 3.

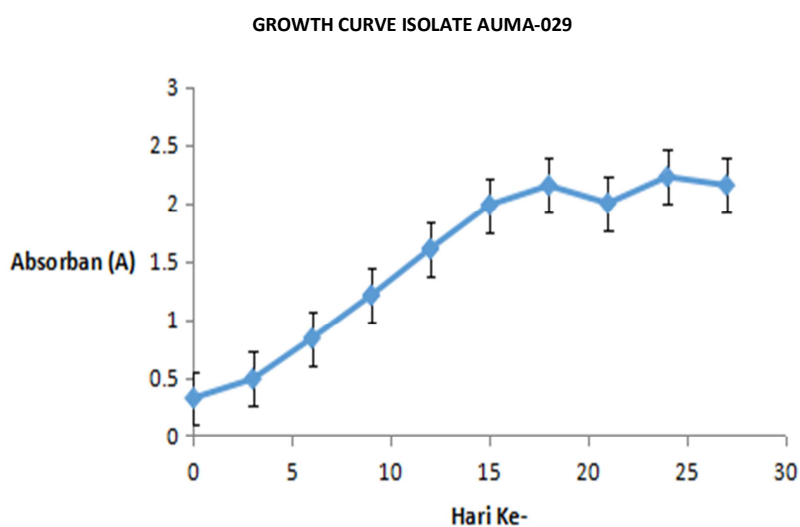


Figure 3. Isolate growth curve AUMA-029 (SD = 0.64573, n = 3)

During cultivation, the culture colour of isolated microalgae (AUMA-029) is green. The more dense the number of cell the more green that it looks, it means the microalgae have a lot of green pigment (chlorophyll)<sup>[16]</sup> in accordance with the results of morphological identification stating isolates AUMA-029 is a type of green alga *Chlorella sp.*

The isolates growth pattern possessed by AUMA-029 starting from the lag phase (adaptation) on days 0-3, followed by a log phase on days 4-15. Metabolism characteristic during log phase is a high photosynthetic activity for protein formation and the composition of the plasma cells needed for growth. This condition is characterized by increasing the culture green color than at the start of culture.<sup>[15]</sup>

Stationary phase cultures experienced at the age of day 16 was marked by the increase of cell relatively fixed. In this phase harvesting is done because in this phase secondary metabolites such as organic carbon most commonly produced. Stationary phase that occurs at day 16-24 phase interspersed with the growth rate decreased on day 21, the phase of growth rate decline usually occurs when nutrients, pH, and CO<sub>2</sub> contained in the medium began to reach its limits.<sup>[17]</sup>

Culture began to reach a phase towards death that is on day 25, seen in this phase the amount of precipitation and culture change color to yellow. The cell death caused by nutrient exhaustion and accumulation of specific metabolic or toxic materials.<sup>[15]</sup>

### Phytochemicals profile

Phytochemical test against these secondary metabolites are performed for samples that have antimicrobial effects. The samples are containing hydrogen peroxide, terpenoids and bromo ether and according to previous studies of *Enhalus acoroides*, the compounds were significantly identified as antimicrobial compounds are fatty acid, acrylic acid, halogen aliphatic, terpenes and phenols<sup>[18]</sup>. When compared with the test results of phytochemical that has been done against the microalgae *Chlorella sp.* which extracted with methanol in Table 1.

Table 1. Phytochemicals Test Results Isolate AUMA-029 and *Chlorella vulgaris* (Adhoni et al, 2016)

No.	Secondary metabolites	isolates Microalgae AUMA-029	<i>chlorella vulgaris</i> (Adhoni et al, 2016)
1	flavonoids	-	+
2	phenolic	+	+
3	saponin	+	+
4	steroids	+	+
5	triterpenoids	+	+
6	alkaloids	-	-

There are differences in the results of flavonoids which in previous studies<sup>[19]</sup> was detected the presence of flavonoid compounds in microalgae *Chlorella sp.* while in this study flavonoid compound was expressed negative. This difference could have occurred because of secondary metabolites are not always shared by all organisms. Environmental conditions and the stage of development of an organism will induce the formation of secondary metabolites. Secondary metabolites are not directly involved in the growth and development of an organism or the reproductive system. Secondary metabolites appear and work in defense of life and the process of adaptation of an organism.<sup>[20]</sup>

Bacterial resistance mechanism by phenolic secondary metabolites can be toxic protoplasm, damage and penetrate the cell wall of bacterial cells and precipitate protein. Causing damage to the bacterial cell ends bacterial death. Triterpenoids as antibacterials can react with Porin (transmembrane protein) in the outer membrane of the cell walls of bacteria, forming a strong bond polymer resulting in damage Porin. Damaging Porin which is the doorway to the compound would reduce the permeability of cell walls of bacteria and bacteria will result in nutritional deficiencies, so that bacterial growth is inhibited and even death. Steroids inhibit the growth of bacteria by destroying the bacterial cell membrane<sup>[21]</sup>. While saponins inhibit bacterial growth by lowering the surface tension of the cell wall and when interacting with the bacterial wall, the wall will be broken. When disturbed surface tension, saponin will enter the cell easily and will disrupt bacterial metabolism and eventually death.<sup>[22]</sup>

### Antibacterial activity

Antibacterial activity test performed against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* as well as Gram-negative bacteria, namely *Escherichia coli* and *Salmonella typhi*. The results are presented in Table 2. Observations were made after incubation for 24 hours.

Table 2, The antibacterial activity of isolates AUMA-029 with methanol

Concentration of Extracts	Inhibition Zones			
	<i>E.coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>
10µg / mL	8 mm	10.5 mm	9 mm	10 mm
100µg / mL	10 mm	9 mm	9 mm	10 mm
500µg / mL	12 mm	9 mm	10 mm	11 mm
control + (Amoxicillin 10µg / mL)	10 mm	9 mm	10 mm	10 mm
control -	7 mm	0 mm	7 mm	6 mm

From the measurement results shows that the clear zone on the test bacteria *E. coli*, *S. aureus* and *B.cereus* give the greatest inhibition zone is shown at a concentration of 500 ug / mL while on *S.typhi* the largest inhibition zone has been seen at a concentration of 10 ug / mL. Differences in increasing and decreasing zones of inhibition against the concentration of the extract is due to the component substances that contained in medicinal plants can be mutually weakened, strengthened, improve or change at all. Giving concentrations lower or higher than the concentration required to kill bacteria can also inhibit the antibacterial activity against the antibiotics given so that the bacteria will be resistance. This vulnerability is caused due to bacteria can produce enzymes that can perform antimicrobial inactivation and disruption of cell membrane permeability thus not achieving an effective antimicrobial concentrations in the cell as well as the modification of the molecules in the cell which is the target of antimicrobials. [23]

When compared with previous studies that have been conducted on microalgae *Chlorella vulgaris* were extracted by using methanol in Table 3 shows that the bacteria that form potential barriers are relatively low.

Table 3. Results of Antibacterial Activity of *Chlorella vulgaris* with Solvent Methanol<sup>[18,28]</sup>

No.	Bacteria Test	Inhibition Zones By <i>Chlorella vulgaris</i>
1	<i>E.coli</i> (Syed, 2015)	15 mm
2	<i>S.aureus</i> (Salem, 2014)	17 mm
3	<i>B.cereus</i> (Salem, 2014)	17.5 mm

This low potency can be caused by the extract which has been obtained is crude extracts and effectiveness are small. This small effectiveness presumably because least of the content from active compound has a similar structure to the antibiotics commonly used to inhibit the growth of the test bacteria used, the age of the bacteria and environmental conditions. [6]

Isolates AUMA-029 which is a microalgae *Chlorella sp.* has potential as an antibacterial, because the content of metabolites contained in them either the content of primary and secondary metabolites. The most important antibacterial compounds owned by *Chlorella sp.* which is a mixture of fatty acids known as *chlorellin*, this compound is able to inhibit the growth of Gram positive and Gram negative. The methanol extract of *Chlorella sp.* can inhibit the growth of bacteria *Bacillus cereus* and *E. coli*<sup>[24]</sup>. The mechanism of inhibition of bacterial growth by a fatty acid that is by attacking and destroying the cell membrane of the bacteria causing the lysis of the bacterial cell, and also these fatty acids will reduce nutrient and inhibits cellular respiration. [25]

Besides containing *chlorellin* as an antibacterial agent, *Chlorella sp.* also containing secondary metabolites such as phenolic, terpenoids, steroids and saponins as an antibacterial agent. Chlorophyll that contained in *Chlorella sp.* can also act as an antibacterial compound. Chlorophyll does not have a bactericidal effect (kill microorganisms) but chlorophyll has the ability bacteriostatic (inhibits the growth of microorganisms), but that under the suitable environmental conditions chlorophyll can also have a bactericidal effect. [24]

In Table 3 shows that the negative control also provides an inhibitory effect on *E. coli*, *Staphylococcus aureus* and *Bacillus cereus*. This is because DMSO is toxic to bacteria. *Dimethyl sulfoxide* (DMSO) is an aprotic solvent that can dissolve both polar and non-polar compounds, therefore it is widely used as a solvent on antimicrobial testing [26]. DMSO 1% can kill 2-10% bacteria, therefore it is recommended to use DMSO in low concentrations at the time of dissolving the antibacterial test extract, to not give effect against bacteria inhibition test. Recommended concentration of DMSO is 0.01% - 0.5% . [27]

## CONCLUSION

The research shown the dominant type of microalgae that live in medium BBM which was isolated from Talago Biru, Koto Baru, West Sumatera is a genus *Chlorella sp.* and the best harvesting time for this microalgae is on the day 20 in the stationary phase. Profile phytochemicals result shown the contained in this isolate are positive phenolic, saponin, steroids and triterpenoid, while alkaloids and flavonoids give negative results. The isolate have potential as a an antibacterial, seen from the test results provided by the zone of inhibition against four bacterial isolates tested, but the activity is low.

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41 Pharmaceutics	journal	ISSN 19994923	0.707 Q1	16	37	106
42 Drug Delivery and Translational Research	journal	ISSN 2190393X, 21903948	0.703 Q1	15	52	148
43 Planta Medica	journal	ISSN 00320943, 14390221	0.702 Q1	89	210	697
44 Cancer Nanotechnology	journal	ISSN 18686966, 18686958	0.701 Q1	9	3	30
45 Journal of Pharmacy and Pharmacology	journal	ISSN 00223573	0.686 Q1	89	206	574
46 Journal of Pharmacy and Pharmaceutical Science	journal	ISSN 14821826	0.682 Q1	59	49	139
47 Drug Delivery	journal	ISSN 10717544	0.662 Q1	40	170	155
48 Biological and Pharmaceutical Bulletin	journal	ISSN 09186158, 13475215	0.644 Q1	88	265	967
49 Archiv der Pharmazie	journal	ISSN 15214184, 03656233	0.628 Q1	43	112	302
50 Research in Social and Administrative Pharmacy	journal	ISSN 15517411	0.62 Q1	25	130	244
51 Journal of Natural Medicines	journal	ISSN 13403443, 18610293	0.602 Q2	28	57	333
52 Journal of Texture Studies	journal	ISSN 00224901	0.578 Q2	40	55	143
53 Journal of Liposome Research	journal	ISSN 15322394, 08982104	0.575 Q2	35	38	110
54 International Journal of Clinical Pharmacy	journal	ISSN 22107703, 22107711	0.575 Q2	43	138	455
55 Recent Patents on Drug Delivery and Formulation	journal	ISSN 18722113	0.574 Q2	24	24	65
56 IEEE Transactions on Nanobioscience	journal	ISSN 15361241	0.572 Q2	46	86	149
57 Saudi Pharmaceutical Journal	journal	ISSN 13190164	0.552 Q2	20	154	202
58 Pharmaceutical Biology	journal	ISSN 17445116, 13880209	0.543 Q2	43	262	632
59 International Journal of Cosmetic Science	journal	ISSN 01425463, 14682494	0.534 Q2	41	96	245
60 Journal of Pharmaceutical Innovation	journal	ISSN 18725120	0.524 Q2	17	25	91
61 Journal of Microencapsulation	journal	ISSN 14645246, 02652048	0.511 Q2	59	89	260
62 Pharmaceutical Development and Technology	journal	ISSN 10979867, 10837450	0.508 Q2	43	123	361
63 Current Drug Delivery	journal	ISSN 15672018	0.506 Q2	45	83	220
64 Acta Pharmaceutica	journal	ISSN 13300075	0.485 Q2	41	40	120
65 Statistics in Biopharmaceutical Research	journal	ISSN 19466315	0.482 Q2	6	34	103
66 Asian Journal of Pharmaceutical Sciences	journal	ISSN 18180876	0.464 Q2	11	57	117
67 Open Drug Delivery Journal	journal	ISSN 18741266	0.458 Q2	5	0	1
68 Die Pharmazie	journal	ISSN 00317144	0.428 Q2	47	122	514
69 International Journal of Pharmacy Practice	journal	ISSN 09617671	0.428 Q2	28	88	198
70 Journal of Advanced Pharmaceutical Technology	journal	ISSN 09762094, 01105558	0.427 Q2	15	40	104

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72 Clinical Pharmacology in Drug Development	journal	ISSN 21607648, 2160763X	0.406 Q2	7	67	145
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74 Pharmacognosy Magazine	journal	ISSN 09764062, 09731296	0.376 Q2	19	78	241
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76 Journal of Nanomedicine and Nanotechnology	journal	ISSN 21577439	0.372 Q2	10	0	109
77 Indian Journal of Pharmaceutical Sciences	journal	ISSN 19983743, 0250474X	0.371 Q2	40	120	305
78 Pharmaceutical patent analyst	journal	ISSN 20468962	0.369 Q2	7	13	130
79 Scientia Pharmaceutica	journal	ISSN 00368709	0.367 Q2	23	40	210
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81 Applied and Translational Genomics	journal	ISSN 22120661	0.355 Q2	3	42	55
82 Journal of Excipients and Food Chemicals	journal	ISSN 21502668	0.347 Q2	9	11	53
83 Letters in Drug Design and Discovery	journal	ISSN 15701808	0.338 Q2	20	102	396
84 Acta Poloniae Pharmaceutica	journal	ISSN 00016837	0.335 Q2	26	147	439
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86 Asian Journal of Pharmaceutical and Clinical Re	journal	ISSN 09742441	0.326 Q2	16	453	1222
87 Pakistan Journal of Pharmaceutical Sciences	journal	ISSN 1011601X	0.316 Q2	28	334	628
88 Drug Metabolism Letters	journal	ISSN 18723128	0.312 Q2	20	18	72
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94 International Journal of Pharmacy and Pharmaci	journal	ISSN 09751491	0.282 Q2	24	939	3740
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96 Canadian Pharmacists Journal	journal	ISSN 17151635	0.276 Q2	12	61	265
97 Tropical Journal of Pharmaceutical Research	journal	ISSN 15965996	0.268 Q2	20	308	573
98 Journal of Drug Delivery Science and Technolog	journal	ISSN 17732247	0.254 Q2	31	116	257
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105 Current Pharmaceutical Analysis	journal	ISSN 15734129	0.225 Q3	18	41	128
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113	International Journal of Applied Pharmaceutics	journal	ISSN 09757058	0.194 Q3	5	8	28
114	International Journal of Pharmaceutical Sciences	journal	ISSN 0976044X	0.193 Q3	16	596	1483
115	International Journal of Drug Delivery	journal	ISSN 09750215	0.192 Q3	8	9	106
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117	Pharmaceutical Care Espana	journal	ISSN 11396202	0.189 Q3	8	30	107
118	Farmaceutvski 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
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123	International Journal of Green Nanotechnology	journal	ISSN 19430906, 19430892	0.167 Q3	1	0	13
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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 Sciences	journal	ISSN 09754725	0.14 Q3	6	0	66
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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
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147	Journal of Pharmacy Technology	journal	ISSN 87551225	0.123 Q3	8	42	116
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149	European Journal of Parenteral and Pharmaceutical	journal	ISSN 17406277, 09644679	0.121 Q4	3	0	68
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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 Clinical	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
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163	Anales de la Real Academia Nacional de Farmacia	journal	ISSN 16974271, 1697428X	0.111 Q4	7	39	102
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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 Research	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
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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 Hospitalares	journal	ISSN 02144697	0.104 Q4	3	0	10
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181	Manufacturing Chemist	journal	ISSN 02624230	0.102 Q4	5	16	364

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184 Pharmaceutical biotechnology	journal	ISSN 10780467, 10058915	0.101 Q4	21	0	310
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186 Pharmaceutical Technology Europe	journal	ISSN 01646826	0.101 Q4	14	146	284
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188 PZ Prisma	journal	ISSN 09455566	0.1 Q4	5	30	105
189 Drug Topics	journal	ISSN 00126616	0.1 Q4	7	288	742
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194 Drug Delivery System	journal	ISSN 09135006	0.1 Q4	2	53	90



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5748	1986	125	13.53	78.74	Netherlands
24836	7585	436	15.97	118.83	Netherlands
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10198	3166	478	6.08	48.79	United States
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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
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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
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3690	738	203	4.5	83.86	Switzerland
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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
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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

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8390	1168	484	1.9	63.56 Netherlands
7958	811	331	2.18	64.18 United Kingdo
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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
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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
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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
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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

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943	116	99	1.07	28.58 Spain
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1397	8	152	0.08	20.54 Slovenia
1329	37	476	0.07	8.92 China
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1384	11	37	0.24	32.19 India
6158	232	757	0.31	22.72 Argentina
107	85	512	0.18	35.67 India
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505	13	63	0.17	28.06 Jordan
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418	35	347	0.07	4.35 United States
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672	6	99	0.06	17.68 Czech Republ
1596	19	108	0.16	26.6 Australia
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296	4	186	0.02	5.29 Croatia
777	5	90	0.03	31.08 Spain
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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
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276	6	52	0.11	21.23 Hungary
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51	1	324	0	3.19 United Kingdo

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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, 09737	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, 18167	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
<b>19</b>	<b><i>Der Pharmacia Lettre</i></b>	<b>journal</b>	<b>ISSN 09755071</b>	<b>0.19</b>	<b>Q3</b>	<b>11</b>	<b>539</b>
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, 22297	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
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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

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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 Research	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 (3y)	Cites (3y)	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
<b>807</b>	<b>13602</b>	<b>354</b>	<b>807</b>	<b>0.44</b>	<b>25.24</b>	<b>India</b>
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

Sheet1

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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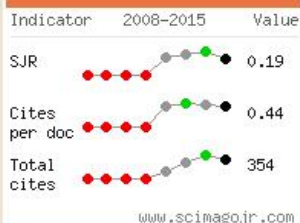
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