Patchouli Alcohol Optimization from *Pogostemon cablin* Benth. cv. Sidikalang Leaves Using Response Surface Methodology

Mochammad Firmansyah[1], Feri Irwansyah[1], Krisyanti Budipramana[1], Mochammad Arbi Hadiyat[2], Ida Bagus Made Artadana[3], Popy Hartatie Hardjo[4*]

Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Surabaya 60293, Indonesia
Department of Industrial Engineering, Faculty of Engineering, University of Surabaya, Surabaya 60293, East Java, Indonesia
School of Bioinnovation and Biobased Product Intelligence, Faculty of Science, Mahidol University, 272 Rama VI Road, Ratchathewi District, Bangkok 10400, Thailand
Faculty of Biotechnology, University of Surabaya. Jl. Raya Kalirungkut, Surabaya 60292, East Java, Indonesia
*Corresponding author: poppy_hardjo@staff.ubaya.ac.id

ABSTRACT
The demand for essential oils in the industrial sector continues to increase, proportional to the number of people using them. *Pogostemon cablin* popularly known as nilam in Indonesia produces patchouli oil with patchouli alcohol as the major compound. Patchouli oil has been used for a long time as perfume ingredients, cosmetics, aromatherapy, insecticides, and pharmaceutical products. Currently, the production process of patchouli oil in Indonesia is not optimal. In order to increase the results of patchouli alcohol, microwave-assisted extraction (MAE) using ethanol 96% as solvent was performed for extraction and analyzed using gas chromatography (GC). Response surface methodology (RSM) statistic was used to calculate the concentration of patchouli alcohol with parameters microwave power (180-600 W) and extraction time (25-60 seconds). The experiment results showed the optimum conditions for extraction were 60 seconds at 600 W with patchouli alcohol (0.23%) and these result similar to patchouli alcohol (0.25%) predicted by RSM for 60 s at 600 W.

Keywords: *Gas chromatography, Microwave-assisted extraction, Patchouli alcohol, Response surface method*

1. INTRODUCTION

Nilam (*Pogostemon* sp.) grows in some regions of Indonesia. Three species of *Pogostemon* lives in Indonesia are *Pogostemon cablin* popularly called Nilam Aceh or Sidikalang because it comes from Aceh or North Sumatera. *P. heyneanus* is known as Nilam Jawa or Girilaya and the last is *P. hortensis* also known as Nilam Jawa but never flowers and the leaves usually used as soap to wash hands and clothes, therefore, *P. hortensis* called Nilam sabun (~ Nilam soap). Regarding essential oil, *P. cablin* ranked first with an essential oil content of 2.5-5% while *P. heyneanus* and *P. hortensis* were in the second rank [1]

As the essential oil from Nilam, Patchouli oil is more concentrated in leaves than in roots and steam. Patchouli alcohol is the major component of patchouli oil in addition to α-bulnesense, α-patchoulene, β-patchuolene, pogostol, pogostone, and eugenol [2]. Patchouli oil is frequently used as base notes in perfumery, cosmetics, foods, beverages, and pharmaceutical products. Since *P. cablin* contains higher essential oil than other species induced *P. cablin* is an economic plant. In addition, *P. cablin* is a unique plant since it has no flowers and the multiplication by vegetative is slow making the price of patchouli oil higher [3].

Patchouli oil is usually extracted using steam distillation with water but the unclean removal of water after extraction induced shorter shelf life thus lowering the price of patchouli oil [1]. Some techniques have been developed to increase the yields of patchouli oil such as pulse electric fields developed by Sukardi et al. [3]. The extraction takes time 8 hours at 2000 volts to yield 2.7% patchouli oil. Kusuma and Mahfud (2015) reported the

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extraction of patchouli oil using microwave for 51 minutes at 634 W and the optimum yield of patchouli oil was 2.80% [4]. Utomo et al. (2017) used supercritical fluid extraction to extract patchouli oil at 135 atm for 252 minutes, yielding 25.34% [5]. To shorten the time and save solvent use, this study aims to optimize the extraction parameters of MAE like time and microwave power and then analyze the yield of patchouli alcohol result using RSM. Response surface methodology is a mathematical and statistical technique used to find the optimum response to the number of factors that affect [6].

2. MATERIALS AND METHODS

2.1 Materials

Eight old weeks of fresh leaves of *P. cablin* (Sidikalang) from the greenhouse (Faculty of Biotechnology, University of Surabaya) were harvested and dried. The determination was performed by Pusat Informasi dan Pengembangan Obat Tradisional (PIPOT) Faculty of Pharmacy, University of Surabaya. Ethanol was purchased from Merck®. RSM was calculated using Minitab 16.

2.2 Extraction process

One gram of grounded leaves of *P.* cablin was extracted using 1 mL of ethanol. A microwave (Samsung® MS30T5018UK) was used to extract with 10 seconds of power on and 20 seconds off for 6 cycles. The microwave power was 180, 300, 450, and 600 W.

2.3 Gas chromatography

Analysis of patchouli alcohol was measured using GC (Hewlett Packard 6890 Series) equipped with Innowax (HP 19095N-123) column. The initial temperature was 71°C (1 min) and the temperature injection was 250°C with a flow rate of 99.7 mL/min. The temperature was programmed from 70°C/min to 240°C. The sample volume was 1 μL with a split ratio of 10:1 and a Flame Ionization Detector (FID). The carrier gas was helium 69 cm/s.

2.4 Response Surface Methodology (RSM)

RSM was known as a design of experiment (DoE) based methodology for optimization purposes. This technique leads to finding the optimum parameters of patchouli alcohol extraction conditions. A factorial DOE was adopted for conducting the experiments and then continued with RSM modeling and response optimization. Each studied parameter consists of 4 to 5-level settings with 45 experiments run under unbalanced replications for each treatment due to equipment operating constraints.

Table 1 shows details of each parameter (or factors) setting as the bases for the experiments. All these levels have considered their effect on the patchouli alcohol extraction process. The area of patchouli alcohol becomes the measured experimental response to be maximized in RSM analysis. The first parameter, i.e. the power of equipment was set in the range of 180 to 600 W, with the pre-assumption that it will significantly affect the response. Similar reason with the second parameter i.e. extraction time was set in 25 to 60 seconds.

Table 1. Parameters (or factors) and levels in the experiment

Factors	**Uncoded levels**				
Power (W)	**180**	**300**	**450**	**600**	
Time (seconds)	**25**	**35**	**40**	**50**	**60**
Measured response: area of patchouli alcohol					

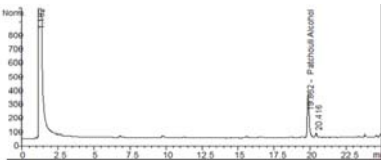
3. RESULTS AND DISCUSSION

Patchouli oil differs essential oil from other essential oil. The uniqueness is due to patchouli oil being rich in sesquiterpenes instead of a mixture of distinct different mono-, sesqui-, and diterpene components. Patchouli alcohol is the main compound of patchouli oil with a sesquiterpene structure and is responsible for the characteristic patchouli base note [7]. This research was conducted to determine the optimal extraction method in order to obtain optimal patchouli alcohol levels. The extraction method selected in this study is MAE by modifying several parameters that affect the extract yield of patchouli alcohol. MAE has been proven to be one of the effective and efficient extraction methods (unconventional) in extracting bioactive compounds from a plant (Ridlo, Kumalaningsih, and Pranowo, 2020) because the faster energy transfer process makes the withdrawal of compounds in the extraction process more optimal so that it can increase the extraction yield and also the extraction process does not take a long time.

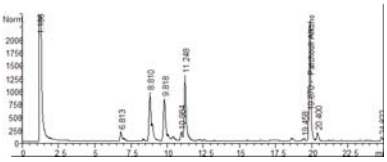
The extraction process using MAE is also influenced by several parameters in the extraction method which can affect the extract yield. The parameters in question include matrix characteristics, solvent volume, power, ekestraction time and also temperature [8]. These five parameters are often used as research variables in order to obtain optimal yield. Research by Kataoka H, 2019 reported that the ratio of solvent, power and extraction time are some of the parameters in MAE that affect the extraction yield. Kusuma and Mahfud (2015) supported this statement and conducted a similar study on this

study, namely the optimization of patchouli oil with MAE and using three parameters such as solvent ratio, power and extraction time. It's just that the difference in the study from this study is related to the research design used in the form of Box Behnken Design (BBD), the value of variations in each parameter point used and the extract solvent used.

First, standard patchouli alcohol GC analysis is required to ensure peak appearance at retention time in the patchouli alcohol chromatogram. Figure 1 revealed the chromatogram of patchouli alcohol extracted using MAE extraction compared to the patchouli alcohol standard. The time retention of patchouli alcohol from MAE was 19.862 minutes like that of the patchouli alcohol standard of 19.870 minutes.



(A)



(B)

Figure 1 Chromatogram of patchouli alcohol from *P. cablin* by microwave-assisted extraction (A) and patchouli alcohol standard (B)

Four microwave power levels (180, 300, 450, and 600 W) were examined with each cycle 10 s power on and 20 s power off and time extraction 25 until 60 seconds. Next, the RSM analysis was used to investigate the parameter effect and their interaction statistically followed by building RSM mathematical model and finished by finding optimum parameter levels to maximize the response based on the result in table 2.

Table 2. Response Surface Methodology (RSM) Analysis Results

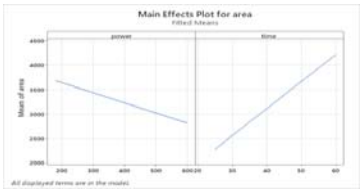
No	Power	Time	Area	No	Power	Time	Area	No	Power	Time	Area
1	180	40	3128.05273	**19**	300	50	2647.06421	**37**	450	60	3548.40186
2	180	40	3890.01001	**20**	300	50	4425.48535	**38**	450	60	4475.75098
3	180	40	4996.91455	**21**	300	50	5398.94238	**39**	450	60	5460.10400
4	180	50	2330.67798	**22**	300	60	1676.80054	**40**	600	25	693.10687
5	180	50	3384.50269	**23**	300	60	2992.72485	**41**	600	25	1024.05298
6	180	50	4382.16748	**24**	300	60	3987.13062	**42**	600	25	1607.62134
7	180	60	2407.31567	**25**	450	25	3389.13892	**43**	600	35	1569.04431
8	180	60	3708.98755	**26**	450	25	988.35864	**44**	600	35	1152.72131
9	180	60	4551.59375	**27**	450	25	1534.19092	**45**	600	35	2044.75891
10	300	25	2626.14575	**28**	450	35	431.73105	**46**	600	40	3028.75545
11	300	25	844.79669	**29**	450	35	790.22473	**47**	600	40	3163.81470
12	300	25	1527.72327	**30**	450	35	864.41095	**48**	600	40	3489.10083
13	300	35	3710.03027	**31**	450	40	4006.39404	**49**	600	60	3231.23869
14	300	35	4742.26807	**32**	450	40	5007.41211	**50**	600	60	3999.42196
15	300	35	3452.07300	**33**	450	40	5366.15479	**51**	600	60	4448.37012
16	300	40	3619.91919	**34**	450	50	2280.89331				
17	300	40	4388.02246	**35**	450	50	3335.78809				
18	300	40	5010.77734	**36**	450	50	4642.29785				

ANOVA analysis indicated information about the significance test with a level of 5% (Figure 2A). Extraction time revealed a significant effect ($P<0.05$) on the concentration of patchouli alcohol while microwave power seems not to influence increasing patchouli alcohol concentration ($P>0.05$). In addition, the slope of time extraction tends to be upright indicating longer time extraction will increase the patchouli alcohol concentration (Figure 2B).

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	3	38633675	12877892	8.54	0.000
Linear	2	30543890	15271945	10.13	0.000
power	1	2784909	2784909	1.85	0.182
time	1	16226044	16226044	10.76	0.002
2-Way Interaction	1	9041990	9041990	6.00	0.019
power*time	1	9041990	9041990	6.00	0.019
Error	41	61834528	1508159		
Total	44	100468203			

(A)



(B)

Figure 2 (A) Analysis of variance (ANOVA) and (B) Main effect plot of each parameter

Moreover, even though only time extraction had a significant influence, interaction between time extraction and microwave power showed significant interaction (Figure 3). This suggested that both parameters interact with each other and cannot be separated to achieve optimum conditions.

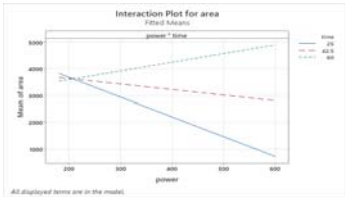


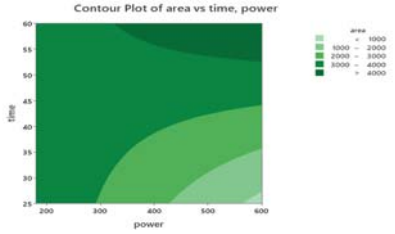
Figure 3 Mean interaction effect of time extraction and microwave power

RSM mathematical model which accommodates interaction both time extraction and microwave power can be seen in equation (1) and by Minitab® the fitted equation is shown in following equation (2).

$$y = \beta_0 + \beta_1 Time + \beta_2 Power + \beta_3 Time * Power \quad (1)$$

$$Area = 6726 - 14.96\, power - 62.9\, time + 0.303\, power * time \quad (2)$$

Interestingly, each of the parameters of time extraction (β_1) and microwave power (β_2) were a negative value and in contrast, the interaction of both parameters gave a positive value (β_3). The interaction of both parameters is more important than the individual because both interactions have proven could increase the area of patchouli alcohol.



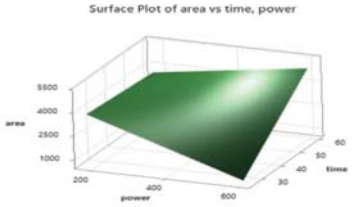


Figure 4 Contour and surface plot of response for fitted RSM equation

This study developed the contour and surface plot RSM from fitted equation (2). Figure 4 displayed the optimal condition setting for time extraction was 60 seconds and microwave power at 600 W with the predicted area of patchouli oil was 0.25%. This prediction aligns with our experiment where the area was 0.23 % at 600 W for 60 s.

AUTHORS' CONTRIBUTIONS

K.B drafted the manuscript; designed the experiments; analyzed the data; **M.F** performed the extraction experiments and collect data; **F.I** performed the extraction experiments and collect data; **M.A.H** drafted the manuscript; designed the experiments; analyzed the data; **I.B.M.A** analyzed the data and corrected the manuscript; **P.H.H** supervised, funding, and resources, corrected the manuscript, and also designed the experiment. All the authors have read and agreed to this manuscript.

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Fenny Irawati, Agnes Natalia Wijaya, Anggi Manurung, Michael Anthony Thongiratama, Wina Dian Savitri, Popy Hartatie Hardjo

The success of tissue culture is greatly influenced by the explant surface sterilization technique. The presence of bacterial and fungi contamination, and the occurrence of browning on the explants can interfere with the process of culture propagation. High concentration of sterilant agents will inhibit...

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Utilization of Tempeh Extract as an Organic Supplement

Alternative for Banana Tissue Culture

Alexander Willy Dimaswarabrata, Anastasia Tatik Hartanti, Listya Utami Karmawan

The addition of organic materials to tissue culture media has been known to have a positive impact on plant growth. However, a tissue culture medium utilizing organic supplements originating from Indonesia as its specialty, such as tempeh, has not been discovered. This study aims to determine the effect...

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Anti-Inflammatory and Mucolytic Activity Test of Ethanol Extract Fennel Leaf (*Foeniculum vulgare* Mill.)

Syifatul Lutviani, Ita Nur Anisa, Andreanus A. Soemardji

Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disease characterized by chronic bronchitis, airway thickening, and emphysema. There are several main mechanisms of COPD, namely chronic inflammatory processes in the airways, oxidative stress, and disturbances in the balance between...

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LFER and 3D-QSAR Analysis of Febrifugine Derivatives against *Plasmodium falciparum* FCR-3 Strain

Nur Aina, Tegar Achsendo Yuniarta, Dini Kesuma

Malaria is a serious disease caused by Plasmodium through the bite of the female Anopheles mosquito. Due to resistance to artemisin, a first-line

antimalarial, new compounds are needed. This study aims to obtain a QSAR model from febrifugine derivatives against the Plasmodium falciparum FCR-3 strain....

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Natural Dyes as Photosensitizers of *Propionibacterium acnes*

Asmiyenti Djaliasrin Djalil, Aqshal Pramudya Susanto, Rizal Nandha Arisugita, Binar Asrining Dhiani, Muhammad Faris Maulidan, Irfan Zamzani

The patient's quality of life may be negatively impacted by the high prevalence of acne vulgaris among adolescents. Acne vulgaris is a skin condition in which hair follicles become clogged with dead skin cells, bacteria, and natural facial oils. It has been demonstrated that acne antibiotics raise antibacterial...

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Formulation of Chewable Gummy Tablet of *Moringa oleifera* L. Leaf Extract Using Combination Kappa Carrageenan and Iota Carrageenan

Nabilaberty Prisma Gemilang, Nikmatul Ikhrom Eka Jayani, Karina Citra Rani

Moringa leaves were the most commonly used part of the Moringa plant because they were rich in nutrients. Moringa leaves extract was developed into a chewable gummy tablet to improve its acceptability. The main component of the chewable gummy tablet was a gelling agent. This study

aims to determine the...

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Stability and antioxidant tests of ethanol extract liposome of moringa leaves (*Moringa oleifera*)

Robert Tungadi, Teti Sutriyati Tuloli, Sri Manovita Pateda

Moringa leaf potentially has an antioxidant effect because it contains Quercetin having poorsolubility in water. Liposomes as carriers of drug compounds can increase the solubility of quercetin through an entrapment system in the lipid bilayer. This study aimed to determine the stability and antioxidant...

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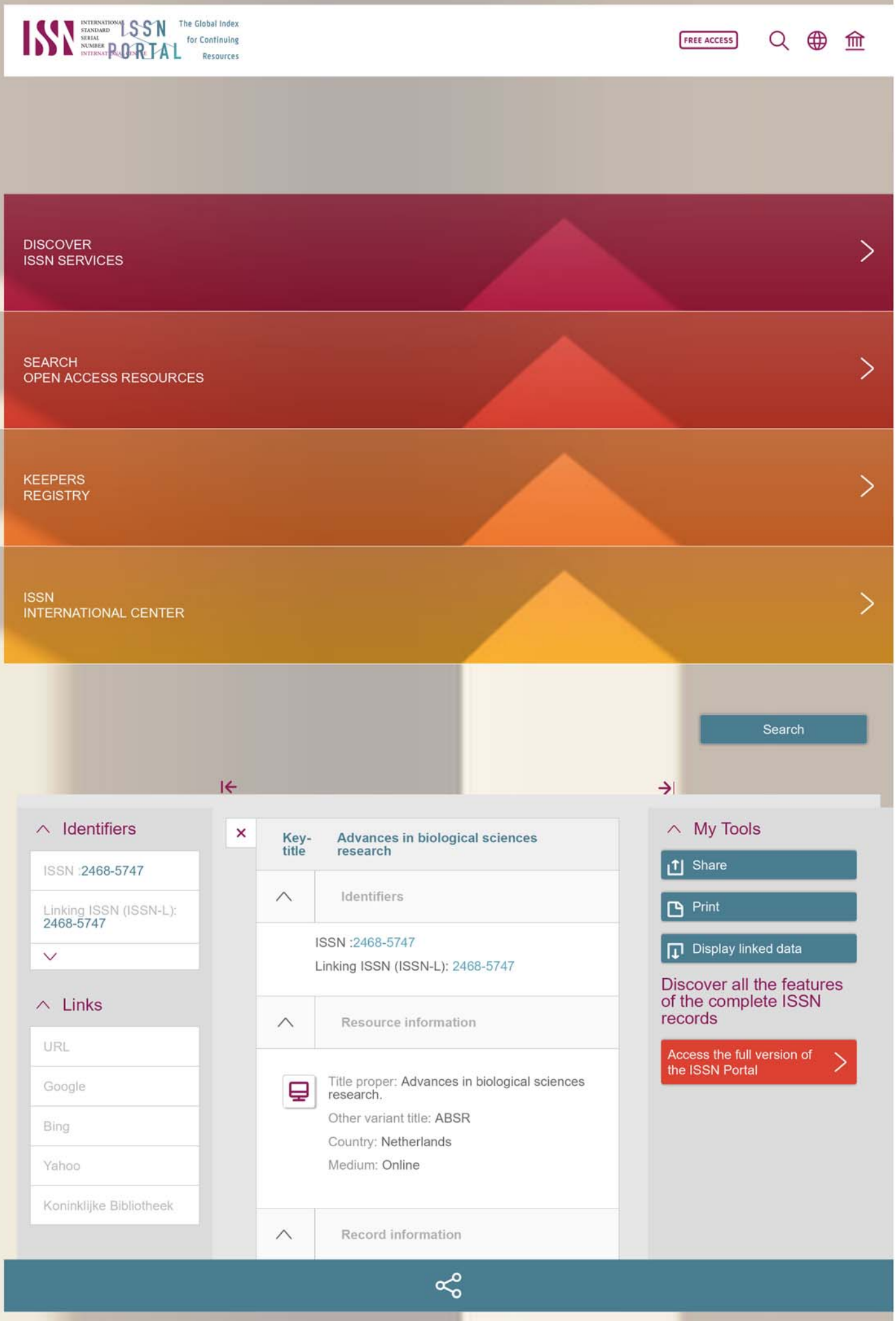
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