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Identification of *Orthosiphon stamineus* from different phytogeographical zones in Indonesia by FTIR-fingerprinting and chemometrics

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ABSTRACT

Orthosiphon stamineus (OS) or cat's whiskers (*Lamiaceae*) is one of the herbs broadly used as raw materials for functional food and traditional remedies, for it contains phenolic acids and flavonoids. OS grows well in tropical and subtropical regions of Asia and Africa. Phytogeographical characteristics are among many variables that greatly control plant growth and phytochemical content. This research was conducted to analyze and classify 14 samples of OS leaves from different regions in Indonesia according to phytogeographic profiles using a combination of Fourier-transform infrared (FTIR) fingerprinting and chemometrics, i.e., principal component analysis (PCA) and cluster analysis (CA). The first two PCs were used to show the grouping of 14 samples with a cumulative variance at 91%. Results showed that all samples had comparable FTIR spectra showing the stretching frequencies of O-H, C-H, and C=C bonds. Further discrimination with PCA and CA was able to classify them into four groups of different phytogeographical origins: group 1 comprised OS samples from Tawangmangu, Mojokerto, Batu, Sampang, Madiun, South Jakarta, Jombang, Surabaya, Kediri, and Ngawi, group 2 from Lampung and Gresik, group 3 from Badung, and group 4 from Kotabaru. Thus, it can be inferred that FTIR fingerprinting combined with chemometrics can distinguish between OS crude drugs from different locations. Introducing a wider variety of OS analytical methods is believed to positively influence the flexibility and ease of quality control of herbal ingredients, ultimately ensuring the safety and efficacy of herbal medicinal products.

1. INTRODUCTION

Orthosiphon stamineus (OS) or cat's whiskers (*Lamiaceae*) is broadly used in traditional medicine by people in Southeast Asia, including Indonesia, Thailand, Vietnam, Malaysia, and Myanmar. Various Indonesian tribes have empirically demonstrated its use in treating rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, gonorrhea, syphilis, kidney stones, nephritis, and gout [1]. Furthermore, different pharmacological tests have confirmed its antioxidant, antidiabetic, antihypertensive, anti-inflammatory, antimicrobial, anti-obesity, diuretic, nephroprotective, hepatoprotective, cytotoxic actions, and activities in the cardiovascular system [2,3]. OS exhibits diverse biological activities due to its various chemical contents, ranging from essential oil, terpenoids, sterols, saponins, and flavonoids to organic acids [2-5].

OS can grow abundantly across phytogeographical regions [6-8]. This ensures a continuous supply of raw ingredients for traditional remedies, both for industrial purposes and self-medication. However, this can be perceived as possible variations in quality, which may create challenges in utilization with standardized requirements. Furthermore, plant quality, which is regulated by chemical compounds, varies according to the geographical conditions of the plant growth site (soil characteristics, climate) and adopted harvesting and post-production systems [9,10]. For these reasons, a straightforward and precise analytical method is required to identify the phytogeographical zone from which an OS sample is collected and assess if its quality is similar or different from other OS samples.

Herbal quality can be evaluated using two analytical approaches: chemical marker identification and compound fingerprinting. A group of markers, i.e., rosmarinic acid, 3′-hydroxy-5,6,7,4′-tetramethoxyflavone, sinensetin, and eupatorin, have been identified using *high-performance liquid chromatography* [5,11], *thin layer chromatography (TLC)* [7], and *high-performance thin layer chromatography* [12-14]. These markers can be used to evaluate OS crude drugs from different locations and OS extracts derived from using varying extraction methods and solvents.

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Compared to these markers, compound fingerprinting offers more advantages. A sample's fingerprint shows a distinctive molecular profile or pattern that reflects and thus provides information on its chemical composition [15,16]. Therefore, in fingerprint analysis, nearly all compounds in a plant sample are considered, allowing a more objective evaluation of its chemical quality than only relying on single or multiple predefined compounds. Several fingerprint-based approaches have been used to detect variations in OS samples collected from different regions, including TLC fingerprinting combined with chemometrics [6], fast gas chromatography-based virtual chemical sensor [8], and FTIR spectroscopy combined with canonical variate analysis [17]. Fourier-transform infrared (FTIR) is a spectroscopic method that efficiently screens medicinal herbs for different chemical compounds. It produces an FTIR spectrum to express the typical chemical bonds of a plant sample. When the peaks in the spectrum change in position and intensity, these shifts correspond to changes in the sample's chemical composition. Therefore, although the chemical makeup of plant samples from the same or closely related species is unknown, FTIR spectra can be used to determine their phytogeographical origins [18,19]. However, visually discriminating chemical compounds from an FTIR spectrum is challenging because the only differentiating factor is the signal intensity. Therefore, it is necessary to combine the fingerprinting process with chemometrics [15].

This research was intended to classify OS leaves harvested from 14 different phytogeographical zones in Indonesia with FTIR fingerprinting followed by two chemometrics, principal component analysis (PCA) and cluster analysis (CA). The observed analytical method is expected to complement previously developed discriminant techniques for more straightforward and flexible quality control of OS leaves as raw materials and in herbal products. Ultimately, a more secure and effective use of OS can be achieved.

2. MATERIALS AND METHODS

2.1. Plant Materials

OS leaves were collected from 14 Indonesian regions in July-October 2020. Details on their phytogeographical origins are shown in Table 1. Samples were the first eight leaves from the top of the shoot picked manually and were verified by the Center for Information and Development of Traditional Medicines, University of Surabaya. On harvesting, the leaves were washed with running water, drained, air-dried, and examined for moisture content using a moisture content balance (Moisture Analyzer HB43, Mettler-Toledo GmbH, Laboratory and Weighing Technologies, Switzerland). Dried leaves were later reduced to powder in a blender (Philips HR 2222, Amsterdam, the Netherlands) and passed through a 45-mesh sieve.

2.2. FTIR Spectroscopy

OS fingerprint profiles were analyzed using an Agilent Cary 630 FTIR Spectrometer equipped with a diamond Attenuated Total Reflectance (ATR) sampling accessory (Agilent Technologies, Inc., CA, USA) and MicroLab software. For each OS sample, the FTIR was read in the wavenumber range of 4000–650 cm^{-1}. Then, the percent transmittance (%T) of each spectral peak at each wavenumber was tabulated, selected, and analyzed using chemometrics.

2.3. Chemometrics Analysis

FTIR fingerprint profiles of 14 OS samples from different phytogeographical regions were analyzed using chemometrics, i.e., PCA and CA, in the Minitab v.16 program (Minitab Inc., USA). In this case, PCA was used to group chemically similar samples by reducing the original variables and replacing them with a number of *n* new variables. PCA was also used to reduce the amount of the inputted data (i.e., FTIR spectra), provided a correlation existed between them. The number of new variables, termed principal components (PC), was determined depending on whether or not the resulting correlation coefficient was significant. Principal component one (PC1) represents the variable with the greatest variation and describes the original variable, while principal component two (PC2) accounts for the next largest variation. If there is a significant correlation between the data, then the number of PCs used will be smaller than the original variables [20].

CA helped group samples according to their similarities into the same group (cluster), starting with considering original variables as the foundation for constructing same-size clusters and then followed by inter-cluster comparisons. Sequential comparison between cluster distances was used to group nearby samples to each other into a new cluster because a shorter separation distance means more similar variables are shared. Then, variables were compared again, and if two other clusters were found to share similarities, they would be categorized into a cluster, and so on, until all clusters became a large one. The derived clusters manifest any similarities and differences between samples.

3. RESULTS AND DISCUSSION

3.1. *Physical Characteristics of* OS *Leaf Crude Drugs*

Physical characteristics of leaf crude drugs (i.e., color and moisture content) are commonly examined as initial parameters in quality evaluation. Figure 1 shows the visual appearances of crude drugs from 14 samples of OS leaves, with their respective moisture contents written in Table 1. All samples had a similar color, dark green. However, a much darker green shade was found in four samples: sample number 4 (from Lamongan), 5 (Gresik), 7 (Ngawi), and 9 (Sampang). Previous research found that methanol extract of OS leaves having a weak green color showed a TLC profile with small chlorophyll bands [6]. In addition, the moisture content of all samples was less than 10%. Moisture content is an important parameter because, in a crude drug, water is a good medium for the growth of microorganisms and insects that can damage the contained chemical compounds [21]. Moreover, the Indonesian Herbal Pharmacopoeia Edition II requires the loss on drying value of OS leaves to be no more than 10% [22].

All samples went through the same harvesting and post-harvesting procedures, so any differences in color and moisture content of the leaf powder can be attributed to their different origins. Samples were also divided into three groups based on elevation: samples from highlands (> 600 masl; consisting of Tawangmangu, Batu, and Mojokerto samples), from lowlands (<200 masl; South Jakarta, Surabaya, Lamongan, Gresik, Jombang, Sampang, Madiun, and Kediri), and medium land, with elevation range lower than highlands but higher than lowlands (200–600 masl; Kotabaru, Badung, and Ngawi). However, when linked to elevation, there seemed to be no direct correlation with the physical characteristics of the leaves; hence, it is predicted that other geographical factors are responsible for the variations.

3.2. *FTIR Spectra of* OS *Leaves*

FTIR spectra of the OS leaf powder were observed directly in the wavenumber range of 4000–650 cm^{-1}. This range can be segmented into

Table 1: Moisture contents of crude drugs from *Orthosiphon stamineus* leaves of different phytogeographical origins.

S. No.	Phytogeographical origins	Latitude, longitude	Elevation (masl)	Moisture content (%)*
1.	Tawangmangu	7°39’50”S and 111°08’04”E	1200	5.66□2.60
2.	South Jakarta	6°15’25”S and 106°46’45”E	7	4.34□1.61
3.	Surabaya	7°16’11”S and 112°44’48”E	7	6.33±1.27
4.	Lamongan	7°06’25”S and 112°20’08”E	7.7	4.86□1.14
5.	Gresik	7°09’58”’S and 113°18’07”E	<200	4.76±1.62
6.	Batu	7°51’52”S and 112°31’12”E	897	3.88□0.31
7.	Ngawi	7°33’27”S and 111°17’38”E	331	4.76±1.62
8.	Jombang	7°36’52”S and 112°22’10”E	62	3.44□0.69
9.	Sampang	7°03’54”S and 113°15’04”E	63	6.46□1.28
10.	Mojokerto	7°38’37”S and 112°36’10”E	650	5.86□2.26
11.	Madiun	7°39’44”S and 111°36’17”E	62	7.67±0.83
12.	Kediri	7°46’07”S and 111°54’36”E	78	3.44□0.40
13.	Badung	8°35’53”S and 115°11’52”E	350	5.16□2.29
14.	Kotabaru	3°17’32”S and 116°13’05”’E	212	4.74±0.55

□mean□SD (*n*□3)

Figure 1: □isual characteristics of *Orthosiphon stamineus* leaf powder.

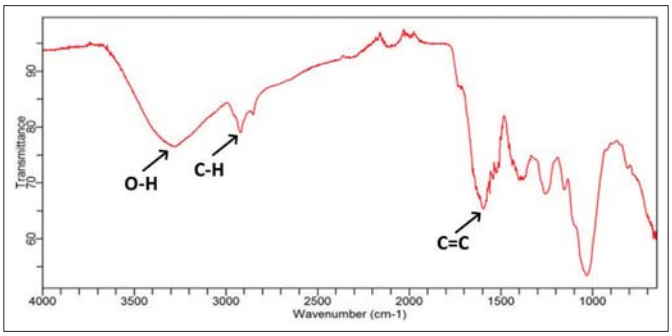

Figure 2: Sample FTIR spectrum of the *Orthosiphon stamineus* leaf from Tawangmangu.

the functional group region from 4000 to 1500 cm^{-1} and the fingerprint region from 1500 to 650 cm^{-1}. Observation of the FTIR spectra in the functional group region was intended to find differences of functional group vibrations of OS leaf samples. However, in that region, FTIR spectra did not show any significant differences in the location and shape of the peaks, indicating a qualitatively similar chemical composition shared by the 14 OS leaf samples. An example is provided in Figure 2. Here, the FTIR spectrum of the Tawangmangu sample shows a broad absorption band at 3278.2 cm^{-1} (the stretching frequency of an O-H bond), the C-H equivalent at 2919.4 and 2851.4 cm^{-1}, and the C=C stretching vibration at 1617.7 cm^{-1}; all are markers of flavonoids and phenylpropanoids [2,17]. The functional groups identified from the 14 OS leaf samples are presented in detail in Table 2.

Afterward, FTIR profiles of 14 OS samples were analyzed using chemometrics. The first step was overlaying the 14 FTIR spectra (Figure 3). Results showed that all spectra had a similar pattern with only a slight difference in the transmittance value of each major or minor peak, indicating identical chemical compounds across the samples. The second step was tabulating wavenumbers against percent transmittances (%T). From these data, 13 peaks in the 4000–650 cm^{-1} wavenumber region were further analyzed using PCA and CA. The data matrix pairing the plant origin with %T at each selected wavenumber of each peak is presented in Table 3.

3.3. *FTIR Fingerprinting with PCA*

PCA is a multivariate analysis used to simplify analysis by reducing the number of intercorrelated variables [20]. In this study, two variables were used: the first principal component (PC1) and the second principal component (PC2). Using two PCs, about 91% variation are described. PC1 accounted for 83.3% of the variation, while PC2 described 7.7% of the variation. This percentage means only 9% of information is lost or, in other words, a significant correlation exists between variables. The proportion and cumulative value of each PC are shown in Table 4, and the scree plot for each PC is presented in Figure 4.

A scree plot displays the relationship between PCs and eigenvalues to demonstrate the amount of variation that each PC collects from the data. The □-axis is eigenvalues, which basically mean the amount of variation, the □-axis is the principal component (PC), and the dots are the proportions shown in Table 4. An ideal curve is steep and then bends at the □elbow□before flattening out. Therefore, two PCs (PC1 and PC2) are sufficient to describe the variance of data in this research.

A loading plot (Figure 5) shows the degree of correlation between each variable and how strongly each characteristic can affect PC.

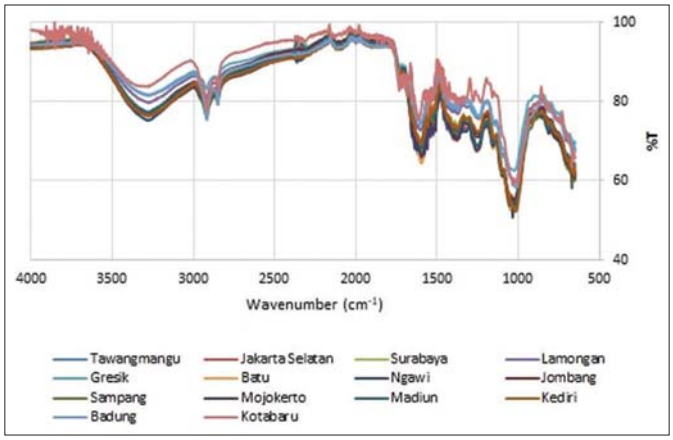

Figure 3: FTIR spectra overlay of the 14 *Orthosiphon stamineus* leaf samples.

Table 2: Functional groups interpreted from FTIR spectra of *Orthosiphon stamineus* leaves harvested from 14 phytogeographical regions.

S. No	Phytogeographical origins	Wavenumbers (cm^{-1})		
		O-H (Alcohol)	C-H (Alkane)	C=C (Alkene)
1	Tawangmangu	3278.2	2919.4 and 2851.4	1617.7
2	South Jakarta	3284.7	2918.5 and 2851.4	1626.9
3	Surabaya	3277.3	2922.2 and 2849.5	1617.7
4	Lamongan	3273.5	2917.6 and 2850.5	1617.7
5	Gresik	3269.8	2918.5 and 2851.4	1601.8
6	Batu	3274.5	2920.4 and 2850.5	1595.3
7	Ngawi	3281.9	2920.4 and 2849.5	1610.2
8	Jombang	3278.2	2920.4 and 2851.4	1602.8
9	Sampang	3276.3	2918.5 and 2849.5	1617.7
10	Mojokerto	3274.5	2917.6 and 2851.4	1625.1
11	Madiun	3273.5	2918.5 and 2850.5	1617.7
12	Kediri	3277.3	2917.6 and 2849.5	1617.7
13	Badung	3270.7	2918.5 and 2850.5	1598.1
14	Kotabaru	3334.1	2916.6 and 2849.5	1608.3

The narrower the angle formed between two vectors, the stronger the positive influence a variable has on a PC, whereas an angle closer to 180° indicates a stronger negative correlation. In contrast, an angle closer to 90° means it is more likely that the two are not related. For example, as seen in Figure 5, the peaks at the wavenumbers 2919.437 cm^{-1} and 1257.977 cm^{-1} show no correlation between the two, whereas those at 2919.437 cm^{-1} and 1700.598 cm^{-1} have a positive correlation.

Table 5 shows the eigenvectors used to calculate the first component (PC1) and second component (PC2) values. These values were then inputted into a score plot (Figure 6). The plot's Euclidean distance was calculated to describe the closeness between samples. Figure 6 shows that OS samples number 13 and 14 have a far Euclidean distance from other samples, while OS samples number 4 and 5 are close together, and other samples (in blue circles) are also close to each other. Therefore, OS samples from 14 locations were grouped into four groups: group 1 containing samples from Tawangmangu, Mojokerto, Batu, Sampang, Madiun, South Jakarta, Jombang, Surabaya, Kediri, and Ngawi; group 2 from Lamongan and Gresik; group 3 from Badung; and group 4 from Kotabaru. This grouping does not appear to be directly related to the elevation of the plant's growing sites. Other factors such as soil type and cultivation process also play a vital role in shaping the quality of medicinal herbs. The results of the current study are consistent with Royani *et al*. (2014), stating that the andrographolide content of □*n*□*ro*□*raphis pani*□*ulata* varies according to its origin and is not related to elevation [23]. Similarly, variations in sinensetin levels of cat's whisker leaves are unrelated to their elevation [7].

3.4. *FTIR Fingerprinting with CA*

CA was conducted after PCA to divide objects into clusters, such that objects with similarities are assigned to the same cluster [24]. It was also to anticipate any failure in PCA's sample grouping. Moreover, CA can provide numerical values of similarity among the objects evaluated. The principle of CA is to combine two closest data points to form a new cluster [20]. The results of the amalgamation step in CA are shown in Table 6.

CA is a method used to group objects based on similarities between variables. It considers original variables as the foundation for constructing a same-size □cluster□ and then compares distances

Table 3: Matrix of the samples' phytogeographical origins versus %T at chosen wavenumber.

Phytogeographical origin	%T value at the selected wavenumber (cm^{-1})												
	3278	2919	2851	2300	2100	1900	1701	1618	1401	1258	1154	1100	1034
Tawangmangu	76.57	78.96	82.14	92.88	95.05	95.11	86.01	66.65	70.60	68.26	69.05	62.86	54.02
South Jakarta	77.03	79.15	82.28	92.93	94.50	94.78	87.94	68.50	72.73	72.31	70.85	61.94	55.72
Surabaya	76.39	78.33	82.12	92.83	94.85	95.15	87.53	69.92	74.16	72.36	71.56	62.96	53.01
Lamongan	79.87	80.78	83.46	93.10	94.34	94.62	88.86	73.39	77.37	75.99	74.84	66.99	60.09
Gresik	81.51	83.24	85.61	94.26	95.29	95.76	89.15	75.34	78.31	76.30	75.00	68.24	62.72
Batu	76.95	78.33	81.60	93.49	95.15	95.25	86.95	67.05	70.49	67.37	68.03	61.04	51.90
Ngawi	75.28	76.79	80.84	92.05	94.24	94.62	88.42	67.60	71.36	72.43	70.60	61.41	51.97
Jombang	77.16	78.91	82.86	93.42	94.91	95.32	86.56	68.45	72.84	71.12	70.73	62.55	54.67
Sampang	76.52	76.08	79.48	92.11	94.03	94.48	85.44	68.83	71.66	68.99	69.23	61.79	52.99
Mojokerto	76.46	78.05	80.99	92.01	93.88	94.35	85.57	68.38	70.84	67.62	68.70	62.66	53.88
Madiun	77.50	77.42	80.83	93.08	94.64	94.82	86.70	70.20	73.29	68.96	69.31	62.97	53.84
Kediri	76.35	78.34	81.18	91.60	93.62	93.85	87.04	70.38	73.63	72.69	70.79	62.92	53.43
Badung	81.71	75.48	79.37	92.53	94.02	94.35	86.39	75.66	78.95	76.98	74.39	66.31	60.16
Kotabaru	84.02	78.18	82.94	95.84	97.37	96.74	85.89	76.76	81.94	82.05	82.35	72.01	59.03

Table 4: Correlation matrix showing eigenvalues, proportions, and cumulative proportions derived from PCA.

Eigenvalue	79.705	7.328	4.099	2.830	0.728	0.569	0.198	0.077	0.051	0.037	0.013	0.005	0.000
Proportion	0.833	0.077	0.043	0.030	0.008	0.006	0.002	0.001	0.001	0.000	0.000	0.000	0.000
Cumulative	0.833	0.910	0.953	0.982	0.990	0.996	0.998	0.999	0.999	1.000	1.000	1.000	1.000

Table 5: Eigenvectors derived from PCA of 14 *Orthosiphon stamineus* leaf samples with different origins.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC 8	PC9	PC10	PC11	PC12	PC13
3278.194	0.278	−0.015	0.136	−0.383	0.192	0.334	−0.136	0.623	0.208	−0.073	−0.265	−0.289	0.011
2919.437	0.070	0.645	−0.193	0.042	−0.248	−0.193	0.149	0.388	0.145	0.117	0.435	−0.066	−0.199
2851.413	0.084	0.470	−0.322	0.000	−0.102	0.013	0.272	−0.138	−0.145	−0.134	−0.656	−0.030	0.306
2299.767	0.083	0.062	−0.239	−0.285	0.065	0.421	0.037	−0.047	0.060	0.221	−0.013	0.742	−0.250
2100.355	0.058	0.001	−0.285	−0.260	0.060	0.272	−0.030	−0.089	−0.140	−0.270	0.529	−0.052	0.622
1900.011	0.043	0.037	−0.209	−0.193	0.061	0.262	0.036	−0.253	−0.496	−0.027	0.073	−0.460	−0.560
1700.598	0.032	0.258	−0.052	0.476	−0.121	0.416	−0.691	−0.128	0.121	0.021	−0.040	−0.070	0.014
1617.665	0.364	−0.077	0.368	−0.010	−0.581	0.092	−0.002	0.128	−0.510	0.255	0.008	0.107	0.164
1400.547	0.397	−0.116	0.111	0.030	−0.339	0.232	0.356	−0.374	0.552	−0.189	0.070	−0.155	−0.109
1257.977	0.453	−0.219	−0.243	0.563	0.253	−0.000	0.178	0.288	−0.208	−0.324	0.050	0.171	−0.108
1153.611	0.409	−0.192	−0.407	−0.020	0.144	−0.238	−0.114	−0.124	0.128	0.666	−0.019	−0.206	0.150
1100.497	0.343	0.022	−0.093	−0.347	−0.152	−0.487	−0.480	−0.131	0.003	−0.431	−0.049	0.173	−0.164
1034.336	0.344	0.432	0.524	−0.002	0.552	−0.050	0.040	−0.283	−0.080	0.074	0.113	0.060	0.060

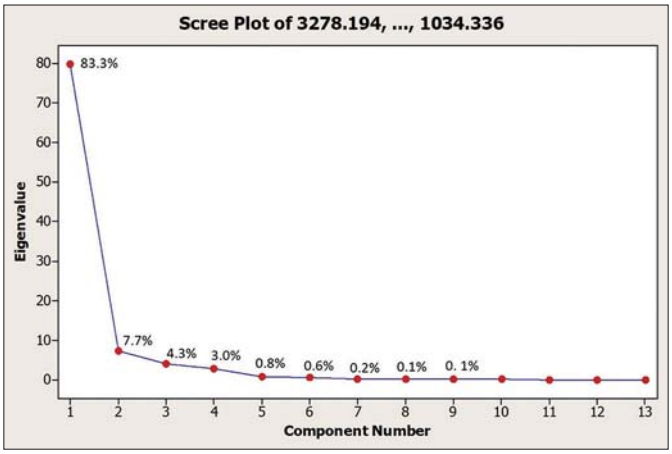

Figure 4: Scree plot of the thirteen principal components obtained from PCA.

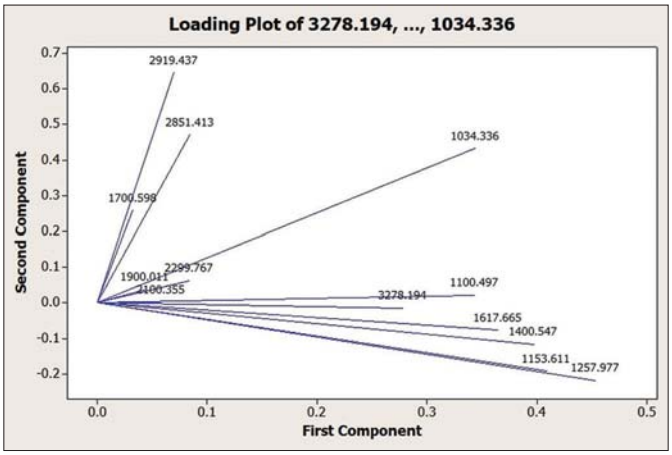

Figure 5: Loading plot of PC1 dan PC2 for the 14 *Orthosiphon stamineus* leaf samples collected from different phytogeographical zones.

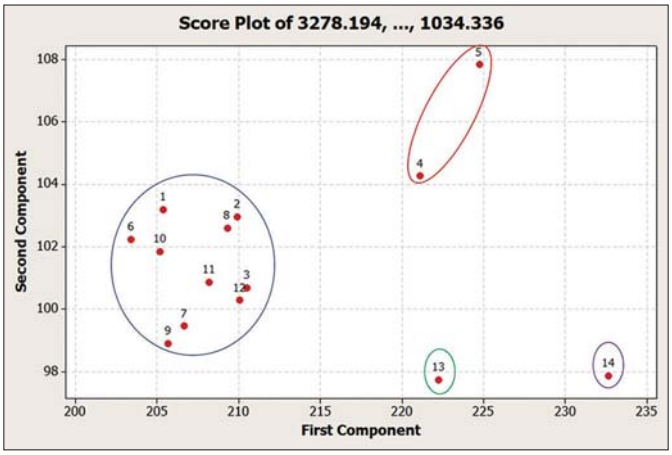

Figure 6: Score plot of PC1 and PC2 from 14 samples of *Orthosiphon stamineus* leaves harvested from different locations. Number 1-14 denotes the sample origin: Tawangmangu, South Jakarta, Surabaya, Lamongan, Gresik, Batu, Ngawi, Jombang, Sampang, Mojokerto, Madiun, Kediri, Badung, and Kotabaru.

between clusters. In the analysis, samples with the same variables are brought together to form a new cluster; then, if further comparison finds two clusters with similarities, they will be merged into one cluster, and so on. As shown in Table 6, the first step was to merge samples from South Jakarta (2) and Jombang (8) with a separation distance of 2.4387 to form one cluster that shared 91.79% similarity. In the second step, samples from Surabaya (3) and Kediri (12) were grouped to form a cluster with a separation distance of 2.6860 and 90.96% similarity. This process continued until the thirteenth step, which combined samples from Tawangmangu (1) and Lamongan (4) into a cluster with a separation distance of 29.7066 and 0.00% similarity. The final results divided samples into four major clusters according to their similarities and differences.

Table 6: Results of the amalgamation steps in the cluster analysis of 14 *Orthosiphon stamineus* leaf samples with different origins.

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	13	91.7907	2.4387	2	8	2	2
2	12	90.9583	2.6860	3	12	3	2
3	11	90.0501	2.9558	1	10	1	2
4	10	87.2971	3.7736	9	11	9	2
5	9	86.4609	4.0220	1	6	1	3
6	8	85.0851	4.4307	2	3	2	4
7	7	81.4683	5.5051	4	5	4	2
8	6	81.0799	5.6205	2	7	2	5
9	5	80.9868	5.648	1	9	1	5
10	4	71.6058	8.4349	1	2	1	10
11	3	62.3782	11.1761	4	13	4	3
12	2	51.6905	14.3511	4	14	4	4
13	1	0.0000	29.7066	1	4	1	14

As shown in Figure 7, the dendrogram grouped the 14 OS samples (□-axis) into four clusters by considering the separation distance (□-axis). The first cluster comprised OS samples from Tawangmangu, Mojokerto, Batu, Sampang, Madiun, South Jakarta, Jombang, Surabaya, Kediri, and Ngawi. The second cluster included samples from Lamongan and Gresik, while the third and fourth contained samples from Badung and Kotabaru, respectively. Sample grouping with CA corresponds to the one generated with PCA. The cluster division is related to similarity in the contained chemical compounds, as observed from the peaks in the FTIR spectra. When associated with elevation, both PCA and CA resulted in random clustering. This can be attributed to many variables related to the conditions of the growing site that cannot be controlled, such as soil type, environmental humidity, rainfall, sunlight intensity, plant habitat, and plant age.

From the study results, it can be concluded that fingerprint profiles obtained using FTIR with the help of PCA and CA (chemometrics) can identify OS samples from different locations and group them into one cluster based on similarities in their chemical compositions. Therefore, fingerprints provide crucial information for when there is a shortage of OS supplies in an area at a particular time or condition. In this situation, OS with the same fingerprint profile grown in other phytogeographical zones can be used. Therefore, based on the PCA and CA results, OS samples of different origins that are assigned to the same cluster can be used as an alternative. This study, however, has some limitations in that the number of samples used was only 14. This widely opens the opportunity for further research to increase the sample size. In addition, it is imperative that other geographical attributes be entirely identified to help determine variables responsible for sample grouping.

The results of this research will also further strengthen previous researches involving FTIR fingerprinting for OS quality evaluation. For example, Heryanto *et al*. evaluated OS collected from 5 locations on Java Island (Indonesia) using transmission spectroscopy techniques, and then the FTIR spectra of the samples were analyzed using PCA and PLSDA [25]. Meanwhile, □uliantini *et al*. used transmission spectroscopy to make FTIR-fingerprinting of OS and its adulterant namely □*upatorium riparium*, then used PCA to differentiate between the two [26]. Furthermore, Ahda *et al*. used FTIR with reflectance spectroscopy technique (ATR FTIR) to differentiate OS extracted with ethanol at various concentrations (20,40, 60, 80, and 80%) [27].

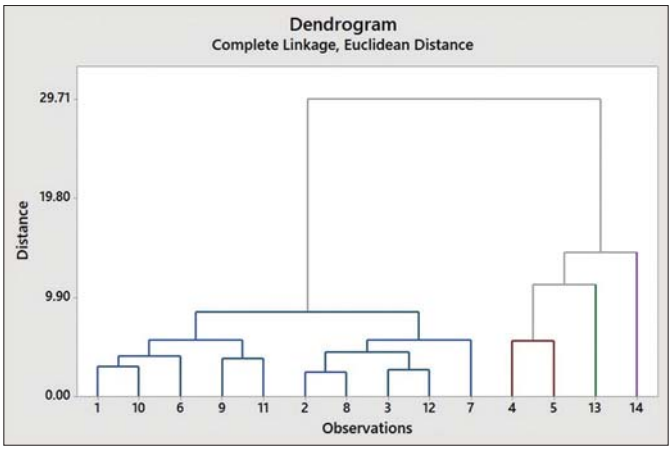

Figure 7: Dendrogram obtained from cluster analysis with complete linkage. Number 1–14 denotes the sample origin: Tawangmangu, South Jakarta, Surabaya, Lamongan, Gresik, Batu, Ngawi, Jombang, Sampang, Mojokerto, Madiun, Kediri, Badung, and Kotabaru.

4. CONCLUSIONS

The FTIR spectra of 14 OS leaves samples show the stretching frequencies of O-H, C-H, and C□C bonds, all are markers of flavonoids and phenylpropanoids. FTIR-fingerprinting combined with chemometrics (PCA and CA) proves to be effective in discriminating between crude drugs of OS leaves harvested from different phytogeographical regions. The first two PCs were used to show the grouping of 14 samples with cumulative variance at 91%. Using this method, OS samples from 14 locations in Indonesia were classified into four groups: group 1 containing samples from Tawangmangu, Mojokerto, Batu, Sampang, Madiun, South Jakarta, Jombang, Surabaya, Kediri, and Ngawi; group 2 from Lamongan and Gresik; group 3 from Badung; and group 4 from Kotabaru. Introducing diverse analytical methods to analyze OS is expected to promote the flexibility and ease of quality control of this herbal ingredient, which, in the long run, will ensure the safety and efficacy of herbal medicinal products.

5. ACKNOWLEDGMENTS

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6. AUTHORS' CONTRIBUTIONS

Kartini Kartini conducted the research design, data and statistical analysis, drafting manuscript, critical revision of manuscript, funding the research, supervision, as well as final approval. Khusnul Khotimah conducted the data acquisition, data analysis, and technical support. Nikmatul Ikhrom Eka Jayani and Finna Setiawan conducted the data acquisition, data analysis, critical revision of manuscript, as well as final approval. Nina Dewi Oktaviyanti and Mochammad Arbi Hadiyat conducted data analysis, critical revision of manuscript, as well as final approval.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

10. PUBLISHER'S NOTE

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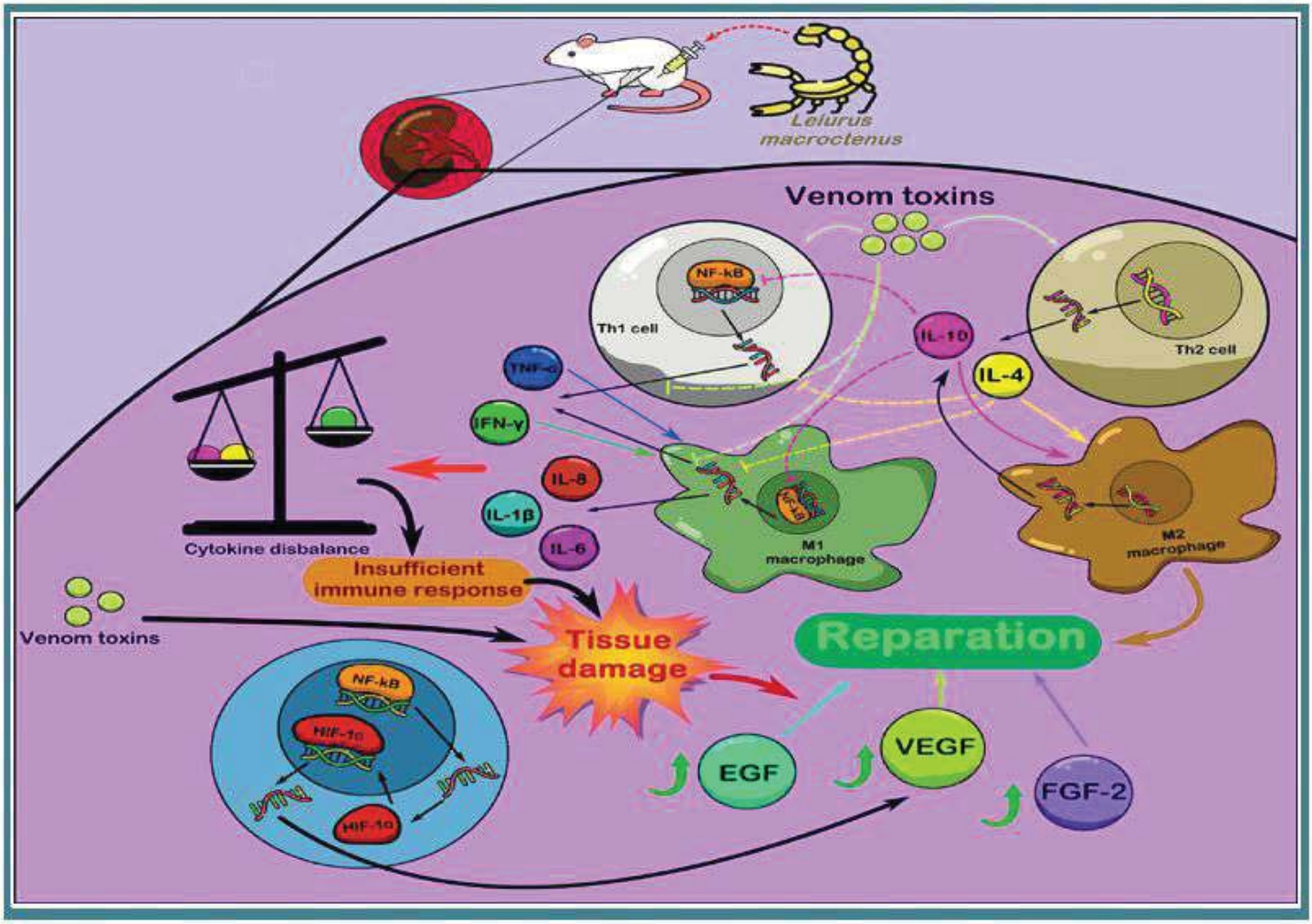

On The Cover: The effects illustrative summary of Leiurus macroctenus venom in the context of inflammation in kidneys of envenomated rats (Image Credit: Matkivska, *et al.*, Clinical Anatomy, Bogomolets National Medical University, Ukraine).

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In this Issue: Mini Review: 1, Research Article: 32, Review Articles: 3

On the cover: The effects illustrative summary of Leiurus macroctenus venom in the context of inflammation in kidneys of envenomated rats (Image Credit: Matkivska, et al., Clinical Anatomy, Bogomolets National Medical University, Kyiv, Ukraine).

Review Articles

20 Apr, 2024

Nanotoxicity: Generation of reactive oxygen species in plants

Mohammad Faizan, Pravej Alam, Vishnu D. Rajput, Ajar Nath Yadav, Shadma Afzal, Sadia Haque Tonny, Ahmad Faraz, Anjuman Hussain, S. Maqbool Ahmad, Tatiana Minkina, Shamsul Hayat

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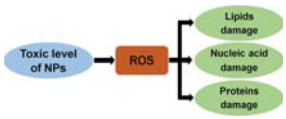

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Antimicrobial peptide and their role in sperm cryopreservation

Manish Kumar, Ravi Ranjan, Alok Bhardwaj

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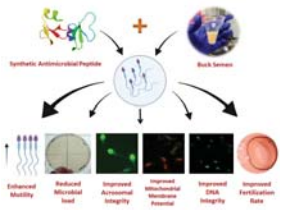

20 Apr, 2024

Impact of nanoparticles on microalgae and the prospects for biofuel production: Current advancements and future outlook

Richa Pahariya, Abhishek Chauhan, Anuj Ranjan, S. K. Thakur, Hardeep Singh Tuli, Seema Ramniwas, Moyad Shahwan, Tanu Jindal

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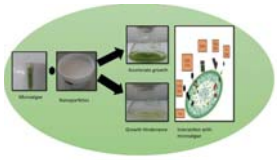

Research Article

20 Apr, 2024

Phenotypic characterization and genetic diversity of the Khiew-Phalee chicken (*Gallus gallus*): A fighting cock originating from Uttaradit, Thailand

Siriwadee Phromnoi, Preeda Lertwatcharasarakul, Wallaya Phongphaew, Pisit Poolprasert

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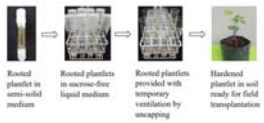
20 Apr, 2024

Improving micropropagation of *Moringa oleifera*: The use of semi-solid medium for rooting and sucrose-free liquid medium combined with temporary ventilation for hardening



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Effects of process parameters on the alcoholic fermentation of pomelo (*Citrus grandis* (L.) Osbeck) juice

Huynh Xuan Phong, Tran Thi Yen Nhi, Nguyen Ngoc Thanh, Le Dang Truong

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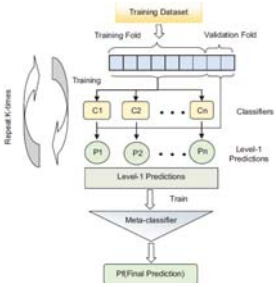

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The matK gene analysis in five types of matoa (*Pometia pinnata* J. R. Forst. and; G. Forst) based on the pericarp colour

Farra Ummush Sholiha, Endang Yuniastuti, Sri Hartati

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20 Apr, 2024

Green synthetic photo-irradiated chitin-silver nanoparticles for antimicrobial applications

Navya Kumari Tenkayala, Laxman Vamshi Krishna Kandala, Roopkumar Sangubotla, Rambabu Gundla, Subramani Devaraju

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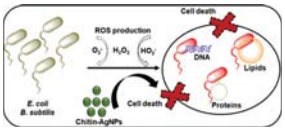

20 Apr, 2024

Cytotoxicity, acute, and sub-chronic toxicity of the *Arctium tomentosum* Mill. root extract

Arailym Aitynova, Nailya Ibragimova, Tamara Shalakhmetova, Karina Vassilyeva, Diana Issayeva

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20 Apr, 2024

Porphyridium sp. Microalgae as a source of polysaccharides

Yulia-Danae Boychenko, Ekaterina Budenkova, Nina Kostyushina, Olga Babich, Egor Kashinskikh

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20 Apr, 2024

Identification of *Orthosiphon stamineus* from different phytogeographical zones in Indonesia by FTIR-fingerprinting and chemometrics

Kartini Kartini, Khusnul Khotimah, Nikmatul Ikhrom Eka Jayani, Finna Setiawan, Nina Dewi Oktaviyanti, Mochammad Arbi Hadiyat

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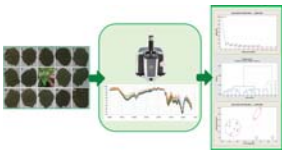

20 Apr, 2024

Evaluation of antibiotic resistance and quorum sensing assay of *Enterococcus faecalis* isolates from the urine samples

Ankita Jaigopal Sagar, Ajay Kumar Oli, Apoorva Jain, R. Kelmani Chandrakanth

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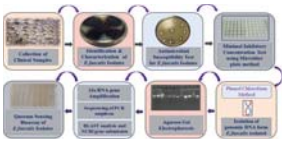

20 Apr, 2024

The arbuscular mycorrhizal fungi inoculation affects plant growth and flavonoid content in tomato plant (*Lycopersicum esculentum* Mill.)

Rusdi Hasan, Tia Setiawati, Dede Sukirman, Mohamad Nurzaman

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20 Apr, 2024

Effect of farmers' knowledge and production practices on the intensity of cassava bacterial blight in Western Kenya

Joe Habert Okoth, James Wanjohi Muthomi, Agnes Wakesho Mwang'ombe

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20 Apr, 2024

Cytokine disbalance in the rats' kidneys following *Leiurus macroctenus* envenomation

Ruzhena Matkivska, Serhii Shchypanskyi, Nataliia Raksha, Tetiana Vovk, Tetiana Halenova, Oleksandr Maievskyi, Olexiy Savchuk, Liudmyla Ostapchenko

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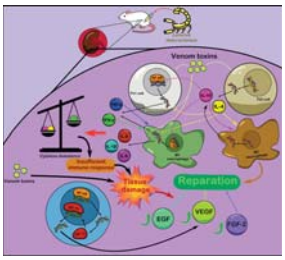

20 Apr, 2024

Evaluation of pancreatic lipase and angiotensin-converting enzyme inhibitors from ethanolic extract of butterfly pea (*Clitoria ternatea* L.) flowers

Ratna Djamil, Diah Kartika Pratami, Tia Novia Wilantika, Qurrata A'yun, Abdul Mun'im, Asep Bayu, Masteria Yunovilsa Putra

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Evaluation of *ex vitro* growth and field performance of micropropagated polyploid clones of *Neolamarckia cadamba*

Wei Seng Ho, Shoufu Gong, Wee Hiang Eng, Kwong Hung Ling

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Performance of mungbean (*Vigna radiata* L. Wilczek) accessions under intermittent water deficit stress in a tropical environment

Uchenna Noble Ukwu, Blessing Ngozika Oburu, Delight Promise Udochukwu, Solomon Oluwaseyi Adewuyi, Stella Ogochukwu Muojiama, Vivian Ogechi Osadebe, Ifesinachi Nelson Ezeh, Patience

Ukamaka Ishieze, Nathaniel Dauda

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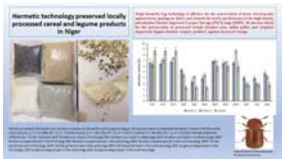
Contribution of the triple hermetic bag in the preservation of locally processed cereal and legume products in Niger



Issoufou Amadou, Ibrahim Boukary Baoua, Abdoulaye Sankhon

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Screening and molecular characterization of phytase producer from north Gujarat region

Madhvi, Hardik Shah

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A low-cost production, characterization, and application of raw starch degrading enzyme from the thermophilic filamentous bacterium, *Laceyella sacchari* P43

Wanthanee Khetkorn, Ausawadee Phonlamai, Chatsuda Sakdapetsiri, Vichien Kitpreechavanich, Thanasak Lomthong

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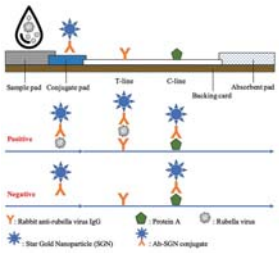
Development and characterization of a lateral flow immunochromatographic assay test strip for rapid detection of rubella virus antigen



Huong Thu Ngo, Tho Tien Ngo, Son Van Tran, Yen Thu Tran, Hien Dang Nguyen, Phong Quoc Truong

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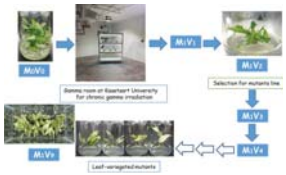
Leaf-variegated mutations induced using gamma irradiation of *Anubias minima*



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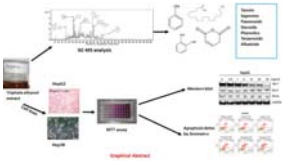
Triphala extract Reduces Anti-apoptotic Protein (Mcl-1) Expression through the Activation of Caspase 3 in Hepatocellular Carcinoma Cells

Yoganchal Mishra, Rohit Raj Yadav, Kavita Verma, Neelam Yadav

DOI: 10.7324/JABB.2024.168741 Pages: 176-184



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Study of morphology and orchid mycorrhizal associations in *Malaxis rheedei*

B. S. Jyothsna, Radha Mahendran, Manish Raj Mishra, Sanjay Dey, Deepti Srivastava

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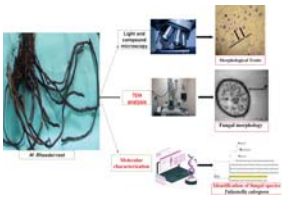

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In silico design of sgRNA for CRISPR/Cas9-mediated FaRALF33 gene mutagenesis to decrease the infection process to *Colletotrichum acutatum* in strawberry

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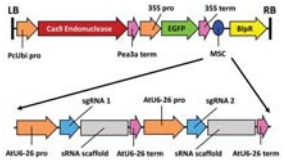

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Genetic dissimilarity, attributes association, and path analysis of sweet peppers

Jannatul Ferdousi, Mohammad Zakaria, Md. Azizul Hoque, Nasrin Akter Ivy, Satya Ranjan Saha, Md. Iqbal Hossain, Shila Pramanik, Dwipok Debnath Dwipok

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AZIN1-centric dynamic network biomarker of tipping point in non-small cell lung cancer metastasis

Anita Chauhan, Kuntal Kalra, Seema Kalra

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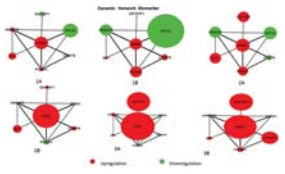

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Agronomic and disease responses of three watermelon (*citrilus lanatus* l.) varieties to fungicide spraying regimes in a tropical environment

Nathaniel Dauda , Sandra Nneamaka Ugwuagu, Patience Ukamaka Ishieze, Kevin Ugwuoke, Vivian

Ogechi Osadebe, Solomon Oluwaseyi Adewuyi, Uchenna Noble Ukwu

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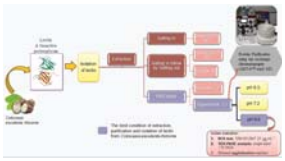
Optimization strategy for purification and isolation of active lectin from *Colocasia esculenta* L. Schott rhizome



Yesi Desmiaty, Chaidir Chaidir, Ariefta Vera Utami, Fauzia Noprima Okta, Gumilar Adhi Nugroho

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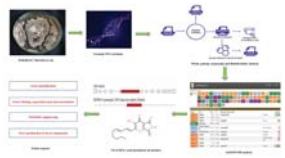

Priyanka N. Shenoy, Sneha Bhaskar, M. Manu, M. P. Likitha, N. Geetha, Shailasree Sekhar, K Ramachandra Kini

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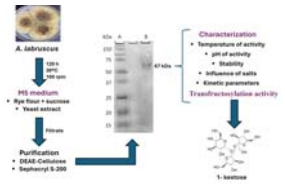

Lucas André Moreira, Arthur Henrique Cavalcanti Oliveira, Luis Henrique Souza Guimarães

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Refined Evolutionary Trees Through an Exceptionally Compatible Alignment-Substitution Model

Ashish Runthala, K. Sowmya, Shamantha Nasika, Bhimavarapu Sai, Atanu Talukdar, Vijayakumar Rajendran, S. Karthikeyan, T. Silambarasan, Manmohan Sharma

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Sustainable biodegradation of textile dye reactive blue 222 by the novel strain Enterobacter CU2004, isolated from the industrial waste: A design of experiment based optimization study and characterisation of metabolites

Vasantha Veerappa Lakshmaiah, Anish Nag, Suresh Gotekar, Sunil More, Shobha K. Jayanna

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Payel Goswami, Sucheta Bhattacharjee, Sudip Bhattacharjee

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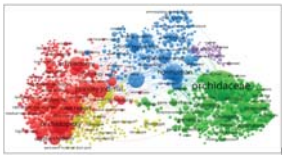

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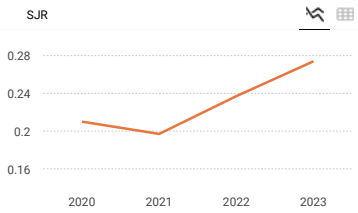

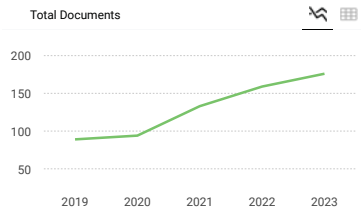

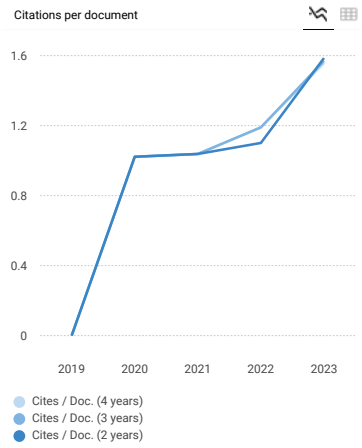

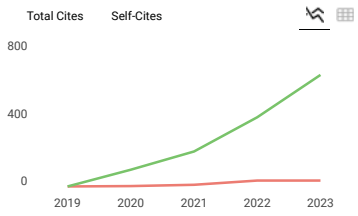

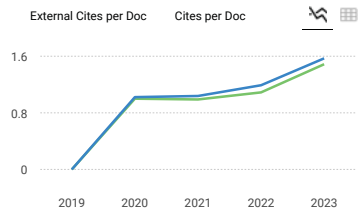

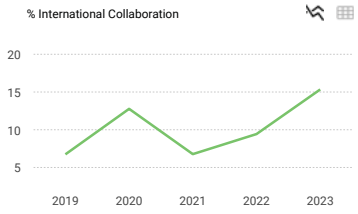

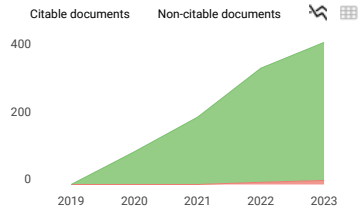

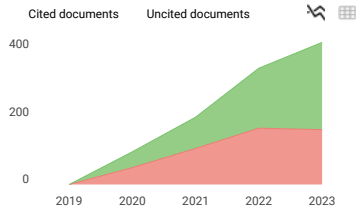

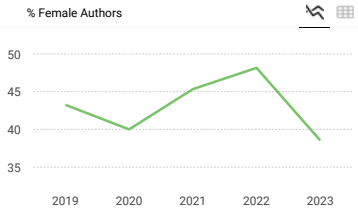

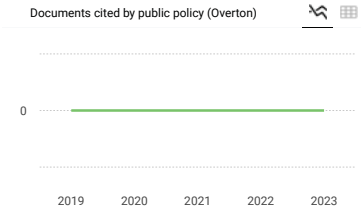

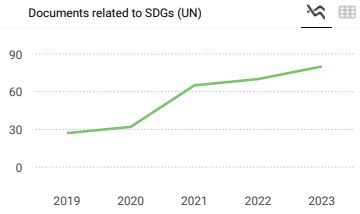

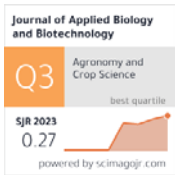

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