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RESEARCH ARTICLE

Metabolite profiling of anticancer compounds in *Saussure lappa* based on UPLC-QToFMS/MS

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Abstract

Background: *Saussure lappa* is a plant of the Asteraceae family that has been known as a medicinal plant to treat cancer. However, there is still a lack of data about the metabolite profiling of this extract and target activity in the cancer signaling pathway. **Objective:** This study aimed to identify metabolites and evaluate the anticancer activity of *Saussure lappa* active ingredients. **Method:** The root sample was extracted using Ultrasound-Assisted Extraction (UAE) with 96% ethanol solvent. Evaluation of the anticancer activity of the compounds was carried out using the PASS SERVER tools. **Result:** About 17 metabolites have been characterised. Interestingly, Saussureamine B, C, D, costunolide, and dehydrocostus lactone showed highly antineoplastic potential properties with several possible activities such as apoptosis agonists, MMP9 expression inhibitors, and Transcription factor NF-kappa B inhibitors. **Conclusion:** The root extract of *Saussure lappa* is highly recommended to be developed as an anticancer drug candidate.

Introduction

Originating from India, Pakistan, and China, *Saussure lappa* (the synonym of *Saussure costus*) is a plant of the Asteraceae family, distributed mainly in the Himalayan region at an altitude of 2,500 – 3,500m (Amara *et al.,* 2017). This plant has other names: Costus (English), Kustha (Sanskrit), Kuy/Kur/Qusthul Hindi (Arabic/Persian), Kostum (Hindi), Potchuk (Tamil), and Kot/Kust (Punjabi language). These herbs annually grow to a height of 1 to 2 meters. This plant requires a cool and humid climate, and the roots are harvested when they reach maximum growth and blossom when they are about five years old (Ansari, 2019).

A previous study found that hydro distilling the roots of *S. lappa* yielded a high concentration of sesquiterpenoids (79.80%), particularly sesquiterpene lactones when compared to monoterpenoids (13.25 %) (Liu *et al.,* 2012; Zhao *et al.,* 2017). However, research conducted by Singh and Shahal (2018) concerning the methanol extract of *Saussure lappa* demonstrated high concentration levels of phenolic and flavonoid compounds, which are 12.34-75.02mg Gallic Acid Equivalent (GAE)/g and 16.2-67.60mg Quercetin Equivalent (QE)/g, respectively (Singh *et al.,* 2018).

Saussure lappa exerts pleiotropic bioactivities such as antifungal (Zahara *et al.*, 2014), antidiabetics (Lammari *et al.*, 2021), anthelmintics (Rao, Babu, & Ramnareddy 2007), antitumor (Lin, Peng, & Su 2015), antiulcer (Hashimi *et al.*, 2020), antimicrobials (Mishra *et al.*, 2018), immunostimulants (Kulkarni 2001; Saif-Al-Islam 2020), anti-inflammatory (Zahara *et al.*, 2014), and hepatoprotection (Tejaswi, Rajan, & Sara 2018). Thus, *Saussure lappa* has great potential to be developed as a phytopharmaceutical.

According to phytopharmaceutical requirements, metabolite profiling is essential to ensure authentication, efficacy, and safety since it can identify the metabolite profile of the raw materials of drugs and final products (Mutiah & Hadya, *et al.,* 2019). However, previous studies have not provided any reports on the metabolite profiling of *Saussure lappa* roots and their antineoplastic properties.

This study is aimed at carrying out metabolite profiling of *Saussure lappa* roots using the UPLCMS/MS method. Evaluation of the metabolite compound's pharmacological activity focusing on the anticancer activity was also analysed using a bioinformatics approach.

Methods

Design

Extraction through the UAE method

An ultrasonic extraction technique extracted fifty grams of Saussurea lappa root powder with 500 ml of 96% ethanol. Each cluster was sonicated for two minutes with three replications. Afterwards, the obtained ethanol extract was evaporated using a rotary evaporator; then it was dried in an oven at a temperature of 40°C until it reached constant weight.

Metabolite identification with UPLCMS/MS

The type of metabolites of the ethanolic extract of Saussure lappa roots was determined using the UPLC-MS/MS instrument. The sample was carefully weighed to reach 10.00mg, dissolved in 10.00ml of methanol, and put into a 5µL microsyringe. The sample was eluted with a mobile phase gradient elution system of water/formic acid mixture (99.9/0.1 [v/v]) and acetonitrile/formic acid mixture (99.9/0.1 [v/v]). The chromatogram of the result of separation through UPLC-MS/MS was processed using Masslynx 4.1 Version application to obtain data in the form of peak area and m/z spectra of each detected peak. Thus, the predicted compounds could be interpreted with the help of the ChemSpider and PubChem websites.

The prediction of pharmacological activity of the Sesquiterpene lactone active compound

The PASS Server investigated the pharmacological activities of each active sesquiterpene lactone. The score listed in PASS Server (Pa) followed these criteria; If Pa > 0.7, the substance was very likely to show activity in the experiment, but the probability of the substance, being an analogue of a known pharmaceutical agent, is also high. If 0.5 < Pa < 0.7, the substance was likely to show activity in the experiment, but the probability is less than the standard chemical substance from the database, and the substance was unlike any known pharmaceutical agent. If Pa < 0.5, the substance is unlikely to show activity in the experiment. Therefore, this study used a cut-off of Pa > 0.519.

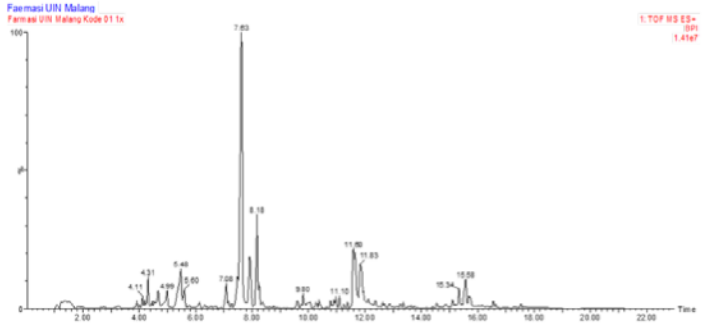
Ethical clearance

This research has received a proper statement of health research ethics from the UIN Malang Health Research Ethical Clearance Commission (No. 114/EC/KEPK-FKIK/2022).

Results

Saussure Lappa extract metabolites as the identification result of the UPLC-QtoFMS/MS method

The metabolite data (Table I) showed that there were 17 compounds as the result of identification, and these compounds belonged to the Glutamic acid, Terpenoid, Flavonoid, Phenol, sesquiterpene lactone, and steroids (Figure 2). The metabolite data also indicate the presence of several dominant compounds or significant compounds, which contain a higher percentage content than the percentage levels of other compounds that the plant contains. The dominant compound is the Sesquiterpene Lactone group consisting of Saussureamine B, Dehydrocostus Lactone, Saussureamine C, and Saussureamine A with the percentage area (38%, 5.47%, 3.9%, and 3.01% respectively). This is also supported by chromatogram data showing that Sausseramine B gives the highest peak at a retention time of 7.63 (Figure 1).

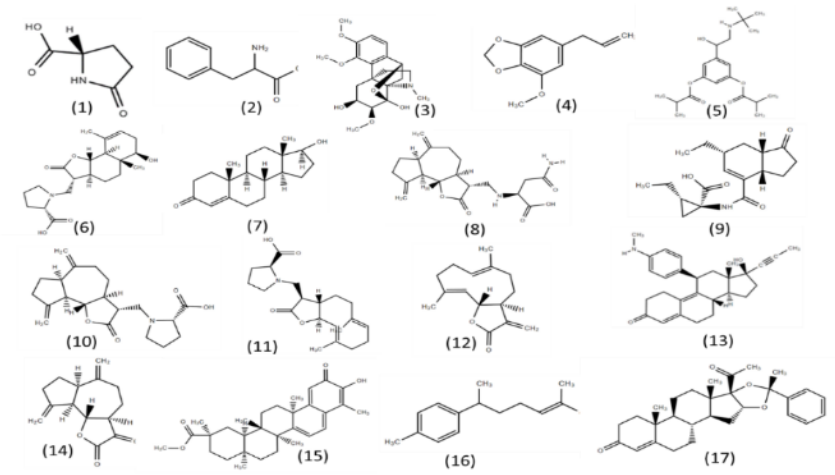


Stationary phase = C18; mobile phase = water/formic acid (99.9/0.1 [v/v]) and acetonitrile/formic acid 99.9/0.1 [v/v]. Each chromatogram peak indicated one compound.

Figure 1: Chromatogram of *Saussure lappa* root extract using UPLC-QToFMS/MS method

The application of Masslynx 4.1 was used to process the chromatogram to find out the m/z spectrum. Therefore, the molecule formula of the interpretation product compound could be predicted. Then, the ChemSpider website helped the researcher find out the prediction's structure and compound name (Figure 2).

After finding out the compound's name and structure, the measured and calculated m/z were compared by drawing the compound structure using Chemdraw Ultra 12.0. If the difference was ≤ 0.0005, then the peak belonged to the predicted compound. The chromatogram data interpretation results are presented in the following Table I.



Structure numbers 6-12 and 14 indicate the sesquiterpene lactone group; terpenoids (3, 13, 15, 16); steroids (7, 17); and Flavonoids (4, 9)

Figure 2: Chemical structure of the *Saussure lappa* root extract metabolite compounds

Table I: The results of metabolite identification of *Saussure lappa* root extract using the UPLC-QtoFMS/MS method

Peak	Retention time (RT)	% Area	Measured mass	Calculated mass	Formula	Compound	Group
1	1.696	0.153%	129.0423	129.0426	$C_5H_7NO_3$	L-Pyroglutamic acid	Glutamic acid
2	2.792	1.297%	165.0790	165.0790	$C_9H_{11}NO_2$	DL-Phenylalanine	Amino acid
3	3.918	1.958%	377.1840	377.1839	$C_{20}H_{27}NO_6$	Stephasunoline	Terpenoid
4	4.002	0.904%	192.0786	192.0787	$C_{11}H_{12}O_3$	Myristicin	Flavonoid
5	5.780	0.316%	365.2201	365.2202	$C_{20}H_{31}NO_5$	Ibuterol	Phenol
6	6.132	1.691%	363.2046	363.2046	$C_{20}H_{29}NO_5$	Saussureamine D	Sesquiterpenes lactone
7	6.554	0.156%	361.2254	361.2253	$C_{21}H_{31}NO_4$	Testosterone 3-CMO	Steroid
8	7.082	3.901%	362.1841	362.1842	$C_{19}H_{26}N_2O_5$	Saussureamine C	Sesquiterpenes lactone
9	7.278	0.583%	319.1788	319.1784	C18H25NO4	Coronatine	Flavonoid
10	7.630	38.010 %	345.1944	345.1940	$C_{20}H_{27}NO_4$	Saussureamine B	Sesquiterpenes lactone
11	8.241	3.011%	347.2099	347.2097	$C_{20}H_{29}NO_4$	Saussureamine A	Sesquiterpenes lactone
12	9.367	0.026%	200.1568	200.1565	C15H20O2	(+)-Costunolide	Sesquiterpenes lactone
13	9.823	2.320%	415.2517	415.2512	$C_{28}H_{33}NO_2$	Metapristone	Terpenoid
14	11.827	5.468%	230.1309	230.1307	$C_{15}H_{18}O_2$	Dehydrocostus Lactone	Sesquiterpenes lactone
15	12.706	1.128%	464.2931	464.2927	$C_{30}H_{40}O_4$	Pristimerin	Terpenoid
16	13.162	0.843%	202.1727	202.1722	$C_{15}H_{22}$	Curcumene	Terpenoid
17	14.091	0.104%	448.2617	448.2614	$C_{29}H_{36}O_4$	Dihydroxyprogesterone Acetophenide	Steroid

Pharmacological activity of the Sesquiterpene Lactone active compound of Saussure lappa extract

Apoptosis is a programmed cell death process that has a crucial role in tissue homeostasis. More evidence indicates that apoptosis is a major target for discovering and developing new anticancer drugs. Thus, many new anticancer agents have been developed from traditional medicines targeting the apoptotic pathway. It has demonstrated beneficial effects in cancer therapy clinically. In addition to the apoptotic pathway, Matrix metalloproteases (MMPs) also play an essential role in tumour growth, invasion, and metastasis. Matrix metallopeptidase-9 has some functions in the migration, invasion, and metastasis of various cancer types through multiple signalling pathways (Liu *et al.,* 2015). Hence, it is necessary to predict pharmacological activity as a basis for further research as presented in Table II. From reading the information in Table II, it can be concluded that Saussureamine B, C, D, costunolide, and dehydrocostus lactone compounds have high antineoplastic effects because Pa > 0.7. Costunolide and dehydrocostus lactone compounds also have high antineoplastic activity in lung and breast cancer.

Table II: The pharmacological activity of sesquiterpene lactone compounds from *Saussure lappa* root extract

Biological process	The pharmacological activity of active compounds					
	Saussureamine A	Saussureamine B	Saussureamine C	Saussureamine D	Costunolide	Dehydrocostus lactone
Antineoplastic	0.602	0.816	0.841	0.731	0.950	0.982
Chemopreventive	0.165	0.151	0.274	0.129	0.440	0.343
Antineoplastic (breast cancer)	0.149	0.203	0.287	0.170	0.669	0.767
Antineoplastic (lung cancer)	0.160	0.444	0.375	0.237	0.752	0.889
Antineoplastic (colorectal cancer)	0.232				0.500	0.137
Antineoplastic (Ovarian cancer)	0.383	0.478	0.402	0.360	0.466	0.583
Antineoplastic (lymphoma)	0.167	0.142	0.128		0.319	0.266
Antineoplastic (brain cancer)				0.220	0.291	
Antineoplastic (squamous cell carcinoma)	0.117	0.333	0.241	0.144	0.239	0.518
Antineoplastic (pancreatic cancer)	0.695	0.613	0.563	0.513	0.545	0.420
Antineoplastic (thyroid cancer)	0.186	0.216	0.197	0.217	0.256	0.282
Apoptosis agonist	0.427	0.307	0.351	0.091	0.902	0.843
Antimetastatic	0.541		0.501	0.507	0.568	
JAK2 expression inhibitor					0.377	0.415
Caspase 3 stimulant				0.275	0.326	0.28
Caspase 8 stimulant				0.248	0.408	0.415
MMP9 expression inhibitor	0.378	0.281		0.340	0.884	0.783
Transcription factor NF kappa B inhibitor	0.415	0.162		0.309	0.756	0.715

Discussion

Metabolite profiling is essential to determine the metabolite profile of the extracts obtained from chemical components with anticancer pharmacological activity. Furthermore, it is also useful for the identification and authentication of marker compounds found in plants so that the extract quality can be controlled. The analytical technique used in metabolite profiling in this study is the UPLC-MS/MS method because it has high resolution, speed, and sensitivity (Mutiah & Bhagawan, *et al.,* 2019). No report regarding metabolite profiling data using the UPLCMS/MS method was found in the previous studies. The findings of this study showed that the root extract of *Saussure lappa* contained 17 metabolites, namely L-Pyroglutamic acid; DL-Phenylalanine; Stephasunoline; Myristicin; Ibuterol; Saussureamine D; Testosterone 3-CMO; Saussureamine C; Coronatine; Saussureamine B; Saussureamine A; Costunolide; Metapristone; Dehydrocostuslactone; Pristimerin; Curcumene; and Dihydroxyprogesterone Acetophenide. This is confirmed by previous studies that reported the presence of costunolid compounds (Kumar *et al.,* 2014), dehydrocostus lactone (Sun *et al.,* 2003),

Saussereamine, A-D (Singh, Chahal, & Singla 2017), and curcumene (Zahara *et al.,* 2014).

The chromatogram profile indicates the authentication of the active compound in Figure 1. This study found that Saussureamine B had the most significant area that is 38%, followed by Dehydrocostus Lactone (5.47%), Saussureamine C (3.9%), Saussureamine A (3.01%), metapristone (2.32%) and Saussureamine D (1.69%). Consequently, Saussureamine B compound can be recommended as a marker compound since it has the highest concentration level and is only specific for *Saussure lappa*. These results also align with the previous studies reporting that *Saussure lappa* contains the sesquiterpene lactone group with a concentration level of 79.80% (Liu *et al.,* 2012), and Sausseramine B is one of the sesquiterpene lactone groups.

The prediction of anticancer activity and molecular mechanism of sesquiterpene lactone compounds are pointed out in Table II. Previous studies have revealed that the active compounds isolated from this plant have medicinal properties, including the main components of sesquiterpene lactone, namely costunolide and dehydrocostus lactone (Rao Vadaparthi *et al.,* 2015); (Malhotra and Singh 2021). Costunolide and dehydrocostus lactone have anticancer, anti-inflammatory, antiulcer, and immunomodulator (Cao *et al.,* 2019). The study conducted by Patel (2020) shows that the dehydrocostus lactone (DHE) compound in *S. lappa* root extract was effective in stimulating the apoptosis of colorectal cancer and breast cancer cells (Patel *et al.,* 2020). Moreover, dehydrocostus lactone and costunolide compounds are also able to inhibit the S-phase development through the pathway of upregulation of Cdk inhibitors and inhibition of cyclin (Kretschmer *et al.,* 2012). This study affirms that the sesquiterpene lactone group has high anticancer activity, particularly in lung and breast cancer. Molecular mechanism prediction (Table II) indicates that the dehydrocostus lactone and costunolid compounds' antineoplastic activity is shown through apoptosis and MMPs. The induction of apoptotic pathways, carried out by these compounds, was predicted through JAK2 expression inhibitors, transcription factor NF kappa B inhibitors, Caspase-3 stimulants, and Caspase-8 stimulants.

Meanwhile, the regulation of the MMPs pathway carried out by these compounds is predicted through MMP9 expression inhibitors. However, the study has several limitations. This study did not use multiple sources for pharmacology activity prediction. The authors did not perform an analysis of active substances with targets in a signalling pathway. Furthermore, in vitro and in vivo studies should be performed to get a more comprehensive view of this plant's anticancer effect.

Conclusion

The findings of this study asserted that the root extract of Saussure lappa contains 17 metabolites. Remarkably, the results of the evaluation of sesquiterpene lactone compounds activity indicated that Saussureamine B, C, D, costunolide, and dehydrocostus lactone compounds had high antineoplastic potential. The prediction of molecular mechanisms showed a high pharmacological activity on apoptosis agonist, MMP9 expression, inhibitor, and Transcription factor NF kappa B inhibitor.

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