Indonesian Journal of Pharmacy

VOL 33 (3) 2022: 434–447 | RESEARCH ARTICLE

Exploring the Potency of Jatropha Seed Meal (*Jatropha curcas*) as a Chemopreventive Agent through Metastatic Inhibition: A Bioinformatics Approach

Ana Fiin Nangimi[1], Ahmad Syauqy Tafrihani[1], Midory Rahmadhany Putri Adisusilo[1] and Riris Istighfari Jenie[1,2]*

1. Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
2. Laboratory of Macromolecular Engineering, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Submitted: 26-01-2022
Revised: 23-06-2022
Accepted: 05-07-2022

*Corresponding author
Riris Istighfari Jenie

Email:
riris_jenie@ugm.ac.id

ABSTRACT

Jatropha (*Jatropha curcas*) is often used as a source of biodiesel owing to the oil content of its seeds. Its processing generates a byproduct (jatropha seed meal) that contains cytotoxic compounds and phorbol esters, which act as cocarcinogens and tumor promoters, respectively. Meanwhile, metastasis is a characteristic of cancer whereby malignant cells spread to another tissue. This study aimed to determine the potential of jatropha seed meal as a chemopreventive agent, particularly an antimetastatic one, using bioinformatics analysis and molecular docking. The predicted target protein was obtained using SwissTargetPrediction. Genecards and the Database for Annotation, Visualization, and Integrated Discovery database were employed to explore the involvement of the predicted target protein in metastatic processes, gene ontology, and biological pathways. The protein-protein network was analyzed using the STRING database and visualized by Cytoscape. Survival analysis of the target protein in cancer was conducted in the Gene Expression Profiling Interactive Analysis database. Jatropha seed meal showed the presence of isoamericanol A, myricetin, daidzein, gallic acid, and rutin. Gene ontology and a KEGG pathway database search revealed proteins that regulated pathways in cancer, microRNAs in cancer, proteoglycans in cancer, and the PI3K-Akt pathway. A total of 11 predicted target proteins correlated to metastatic in extracellular matrix cell component and 20 prediction targets of jatropha seed meal compounds were aligned resulting in 2 potential targets, MMP9 and HSP90AA1. Survival analysis revealed that patients with higher MMP9 expression had shorter overall survival than those with lower expression of the same. We performed molecular docking to a protein involved in metastasis, MMP9 (PDB ID: 6ESM) using MOE software. The results revealed that isoamericanol A, daidzein, and myricetin exhibited a stronger binding affinity than native ligands and other compounds. Altogether, based on our *in silico* study, jatropha seed meal has potential to act as an antimetastatic agent. However, comprehensive *in vitro* and *in vivo* studies are needed to explore the possibility of developing it as an adjunct therapy in combination with a chemotherapeutic agent.

Keywords: jatropha, phorbol esters, bioinformatics, anticancer, metastasis

INTRODUCTION

Cancer remains the most complex and challenging disease to treat and cure in the world. Various plants have been explored in detail for their potency as chemopreventive agents that can halt cancer progression (Huang *et al*., 2020). Meanwhile, metastasis is the process of invasion and migration of tumor cells from the original tissue to other sites of the body. Cancer progression reaches a fatal level when malignant cells migrate to and invade tissues other than their site of origin, a process known as metastasis. This process begins

with local invasion of invasive tumor cells through their attachment to the extracellular matrix (ECM) (Liang *et al*., 2019). The malignant cells move through the connective tissue with its meshwork of ECM proteins and begin to spread (Eble and Niland, 2019). Cancer metastases included several stages, such as the formation of new blood vessels (angiogenesis), cell attachment, invasion, migration, and cell proliferation involving proteolytic enzymes such as matrix metalloproteinases (MMPs) (Liao *et al*., 2012). Metastasis is the most prominent cause of death from cancer (Eble and Niland, 2019). More than 90% of cancer deaths occur due to the spread of tumor cells through metastasis. Therefore, metastasis is one of the main targets of anticancer agents (Robinson *et al*., 2017).

Several reports have shown the potential of *Jatropha curcas* to act in chemoprevention, in particular, as an antimetastatic agent (Muangman *et al*., 2005; Abdelgadir and Van Staden, 2013). Jatropha (*Jatropha* sp.) is a plant belonging to the Euphorbiaceae family, originating from the tropics of Central America and, more recently, has spread to the continents of Asia and Africa (Montes and Melchinger, 2016). Jatropha has been used as a treatment for various diseases and infections for many years (Asuk *et al*., 2015). Previous studies have shown its activity in the treatment of rheumatism, as well as its wound healing, antidiarrheal, antidiabetic, immunomodulatory, and anticancer effects (Sharma *et al*., 2012).

Although jatropha seed is toxic as the main component of biodiesel energy, some of its parts have been used in traditional medicine. Previous reports showed jatropha roots to have a high detoxification capacity; the root bark has antiproliferative activity on a human liver cancer cell line (HepG2), and the leaves are used as antipyretic and analgesic agents (Katagi *et al*., 2017). Jatropha seed is also used for certain purposes, such as treating arthritis and jaundice, wound healing, repairing fractures, healing burns, and purging (Pandey *et al*., 2012). Ethanol extract of jatropha seed meal contains a diamide group, namely caffeoylaldehyde, that possesses antiinflammatory activity (Abdelgadir and Van Staden, 2013; Yao *et al*., 2012). *Jatropha curcas* is widely used in Mexico in the treatment of cancer (Alonso-Castro *et al*., 2011). Curcin in the seed extract has been shown to inhibit tumor cell growth in cellular pulmonary cancer and gastric cancer (Luo *et al*., 2007). Additional findings were that curcusone A and curcusone B from jatropha seed extract also possessed antimetastatic activity (Abdelgadir and Van Staden, 2013). Curcusone A reduced the secretion of MMP and invasion of cancer cells (Muangman *et al*., 2005). Furthermore, curcusone B suppressed MMP2 secretion and cell motility in the cholangiocarcinoma cell line KKU-100 (Abdelgadir and Van Staden, 2013). Another study discovered the presence of curcusone B isolated from the jatropha root (Muangman *et al*., 2005).

Interestingly, contradictory compounds are present in the jatropha seed. Some of its parts are suitable for therapeutic uses but also have a toxic component (Huang *et al*., 2020). Several compounds of jatropha seed revealed cytotoxic activity against cancer cells, but other compounds, such as 12-deoxy-16-hydroxyphorbol, were identified as tumor promoters (Katagi *et al*., 2017). The oil production process from jatropha seeds generates a byproduct called jatropha seed meal, which contains compounds with cytotoxic activity (Marrufo-Estrada *et al*., 2013). Several physical and chemical methods have been developed to remove phorbol esters, which are the predominant toxic compounds in jatropha seed meal and have thermostable properties (Abou-Arab *et al*., 2019). *Jatropha curcas* is well known for the ethnopharmacology of its traditional uses, but most of the information is empirical and lacks scientific validation (Oskoueian *et al*., 2011). Continuous studies have been carried out to determine the bioactivities of *Jatropha curcas*. Jatrophalactone, a diterpene isolated from Jatropha root exerted cytotoxic activity against human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7221, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cell lines with IC_{50} values of 8.5, 20.6, 19.7, 20.1, and 19.2 μM, respectively (Liu *et al*., 2012).

The existence of compounds with anticancer activity in jatropha seed meal makes it a potential adjunct therapy that could inhibit the growth of cancer cells. Therefore, we conducted a bioinformatics study and molecular docking analysis to determine the antimetastatic potential of unique compounds in each part of the jatropha plant, and the predicted protein targets correlated to metastasis. Moreover, we also analyzed the protein regulators of metastasis that could be targeted by the active compounds in jatropha seed. This study aimed to explore the potential of jatropha seed meal as an antimetastatic agent based on *in silico* experiments.

MATERIAL AND METHODS

Data collection

A bioinformatic study was performed on proteins involved in the metastatic process by employing the online database Genecards (https://www.genecards.org) using the default setting and "cancer metastatic" as a correlated keyword (Safran *et al*., 2021). We limited the results to the 1000 top scoring proteins for further analysis.

GO and KEGG pathway enrichment analysis

Gene ontology, especially the cellular component category, and KEGG pathway analysis of proteins contributing to metastasis were evaluated using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8. (Huang *et al*., 2009), with $p < 0.05$ applied as the threshold for significance (Hermawan *et al*., 2021).

Prediction target of jatropha seed meal compounds

Target prediction was carried out by searching the online database SwissTarget-Prediction (http://www.swisstargetprediction.ch) based on the SMILES code of the compounds using the default setting. SwissTargetPrediction is based on the similarity of the user's query compounds with database collections with known activity through experimental binding assays (Daina *et al*., 2019). To identify the potential protein target of jatropha seed meal compounds in cancer metastasis, we eliminated duplication using the online tool Interactivenn (http://www.interactivenn.net) to construct a Venn diagram (Heberle *et al*., 2015).

Protein–protein interaction (PPI) network and hub gene selection

A protein–protein interaction (PPI) network map was constructed by using the STRING-DB v11.5 database (https://string-db.org) to integrate all predicted correlations among proteins. This database gathers evidence by text mining the scientific literature, experimental databases, and computational prediction interactions, as well as systematic transfer of interaction evidence among organisms (Szklarczyk *et al*., 2021). We applied the cutoff criteria of a confidence score >0.4 as the criterion for significance and a maximum number of interactions = 0 (Hermawan *et al*., 2020). Cytoscape software was employed to visualize the PPI network. Furthermore, CytoHubba software was applied to analyze the top 20 highest degree scores to be selected as hub genes (confidence score >0.4, maximum number of interactions >5).

Survival analysis of potential target proteins

A survival analysis of target proteins was performed by searching the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia2.cancer-pku.cn), using median cutoff and cancer datasets including lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), colon adenocarcinoma (COAD), and kidney renal clear cell carcinoma (KIRC). The number of samples is displayed on the curve. Furthermore, the protein expression level was evaluated using the GEPIA database (Tang *et al*., 2017). GEPIA plots expression profiles by applying the transformation |Log2FC| = 2, with a *P*-value < 0.05, a jitter size of 0.4, and matched TCGA normalized to the GTEx data as the cutoff. Gene expression was classified as high and low when the number of transcripts per million was above and below the median, respectively.

Molecular docking

A molecular docking simulation was performed using MOE 2010.10 software (software license from Faculty of Pharmacy, UGM) to confirm the binding activity of Jatropha seed meal compounds against MMP9. The crystal structure for MMP9 was obtained from the Research Collaboratory for Structural Bioinformatics protein data bank using PDB ID: 6ESM. The protein was processed by deleting the ligand and water molecules. Molecular docking analyses were conducted on a Windows 10 operating system, with an Intel Core i5-10th Gen processor and 8 GB RAM. The docking simulation used the default setting. The London dG and triangle matcher were used for the score function and placement setting, respectively. The force field method was employed to refine the docking results of 30 retained settings. The molecular docking result was described as the docking score and ligand interaction of each compound on MMP9.

RESULTS AND DISCUSSION

GO and KEGG pathway analysis of proteins involved in cancer metastasis

This study investigated the molecular mechanism of jatropha seed meal compounds as antimetastatic agents using a bioinformatics approach. Initially, we used the Genecards database to determine the protein elaborated in the metastatic process.

Table Ia. Gene ontology analysis of proteins in the metastatic process using the PANTHER database

Term	*P*-value	Genes
Cell component		
GO:0031012~ extracellular matrix	3.47E-10	SERPINE1, PDGFB, TNC, ILK, HSPB1, THBS1, CLU, LGALS3, LGALS1, TIMP2, FLNA, TIMP3, APOE, RAC1, CTSD, TGFB2, POSTN, HSP90AA1, TGFB1, MMP7, JUP, HSPA5, ANXA2, MMP1, MMP2, TUBB, FN1, COL1A1, MMP11, SFRP1, MMP14, PKM, MMP13, RPS20, VIM, CALR, NES, GAPDH, FGFR2, FBN1
GO:0005578~ proteinaceous extracellular matrix	1.14E-05	SERPINA1, SPARC, FGF1, SHH, LGALS1, GPC3, TIMP2, TIMP3, TIMP1, WNT1, MUC4, POSTN, TGFB1, MMP7, MMP1, MMP2, MMP3, WNT5A, FN1, MMP9, VEGFA, BMP4, MMP11, SFRP1, MMP13, LOX, HPSE, CALR, FBN1
KEGG pathway		
hsa05206: MicroRNAs in cancer	2.13E-115	MIR30B, MIR30A, IRS1, TNC, MIR146A, MIR106B, MIR203A, RASSF1, CCND2, CCND1, MIR155, PLAU, MYC, STMN1, MIR152, CYP1B1, MIR150, EP300, MIR30E, MIR181A2, TP63, MIR30D, MIR181A1, PDGFRB, PDGFRA, MAP2K1, MAP2K2, MIR9-3, MIR15B, MIR9-1, MIR15A, MIRLET7I, MIR27A, PRKCA, MIR133B, MIRLET7G, DICER1, MIRLET7D, CDC25C, MIRLET7E, MIRLET7B, CDC25A, MIRLET7C, MIR324, CCNE1, MIR181B1, EZR, RAF1, TP53, MIR181B2, MIR20A, DNMT1, ABCB1, NOTCH1, SHC1, PDGFB, MIRLET7A3, MIR32, MIR31, MIR206, MIR205, MIRLET7A1, MIR335, MIR214, BCL2L11, MIR331, MIR210, ABL1, MCL1, FZD3, CREBBP, ST14, TGFB2, HMGA2, MIR200A, MIR29C, MIR200B, MIR200C, MIR29A, NFKB1, MIR215, MIR92A1, MIR224, MIR223, MIR222, CDK6, MIR342, MIR100, MIR221, MIR199A1, BCL2, MDM2, MIR183, GRB2, MDM4, ATM, FGFR3, EZH2, MIR29B1, MIR29B2, CDKN1A, MIR10B, CDKN1B, MIR10A, MIR17, MIR34A, ITGB3, PTEN, BRCA1, MIR18A, MIR96, BMI1, MIR107, CASP3, MIR195, DNMT3B, TIMP3, MIR192, HRAS, MIR193B, ABCC1, MIR181C, MIR99A, MIR23B, MIR23A, MIR25, DNMT3A, MIR21, MIR16-2, MIR19A, SIRT1, MMP9, RHOA, SERPINB5, MIR125A, MIR126, ZEB2, ZEB1, MIR449A, MIR122, MIR483, MIR101-1, MIR124-1, ITGA5, SOS1, MIR128-1, MET, MIR128-2, CD44, MIR451A, MIR520C, PTGS2, THBS1, EGFR, MIR137, NRAS, MIR16-1, ERBB3, MIR375, MIR373, ERBB2, E2F1, E2F3, BAK1, CDKN2A, MIR26A1, STAT3, MIR199B, MTOR, VEGFA, MIR423, CYP24A1, MIR145, APC, MIR125B1, MIR143, CCNG1, MIR141, PDCD4, KRAS, VIM, RECK
hsa05200: Pathways in cancer	3.51E-77	RB1, FGF1, ETS1, FGF2, IGF1R, FGF4, EDNRA, RASSF1, FGF7, CCND1, CDH1, MYC, AKT2, AKT3, AKT1, EP300, SKP2, PRKACA, PDGFRB, PDGFRA, MAP2K1, MAP2K2, DAPK1, HGF, WNT5A, MITF, PRKCA, RUNX1, MSH6, AR, MSH2, SMO, MSH3, CCNE1, RAF1, TP53, CSF1R, MAX, EPAS1, PDGFB, TGFA, KLK3, PIK3R2, PIK3R1, HIF1A, FOXO1, ABL1, FADD, WNT1, STAT5A, SMAD2, STAT5B, FZD3, CREBBP, TGFB2, JUN, SMAD4, TGFB1, SMAD3, JUP, PTCH1, FN1, BRAF, IGF1, PTK2, NFKB1, BMP4, NFKBIA, BMP2, IL6, CXCL12, CDK6, CDK4, GNAQ, GNB1, CDK2, BCL2, MDM2, GNAS, CYCS, GRB2, FGFR3, FGFR2, BCL2L1, FGFR1, RET, ITGB1, GSK3B, CDKN1A, CDKN1B, CXCL8, FLT3, PTEN, SLC2A1, FASLG, BRCA2, GLI1, PIK3CG, CASP9, SHH, CASP8, CASP3, SUFU, ITGAV, RAC1, HRAS, JAK1, HSP90AA1, CHUK, DCC, MMP1, MMP2, AXIN1, FOS, AXIN2, MMP9, RHOA, TGFBR1, TGFBR2, PAX8, PIK3CA, KIT, RARA, RARB, BIRC5, ITGA6, PPARG, SOS1, MET, BIRC2, BIRC3, FH, HDAC2, HDAC1, GSTP1, LEF1, XIAP, CXCR4, CBL, PTGS2, RELA, EGFR, CDC42, NRAS, MAPK8, GNA11, ERBB2, E2F1, CTNNA1, MAPK1, E2F3, VHL, BID, MAPK3, NTRK1, TCF7L2, CDKN2B, NOS2, CDKN2A, BAD, STAT1, EGF, STAT3, VEGFC, VEGFD, MLH1, MTOR, PML, VEGFA, KITLG, RAD51, APC, BAX, FAS, CTNNB1, KRAS
hsa05205: Proteoglycans in cancer	7.63E-43	ITGB1, CDKN1A, MIR10B, MIR10A, ITGB3, FASLG, FGF2, TNF, PIK3CG, ACTB, IGF1R, CCND1, PLAU, MYC, CASP3, AKT2, AKT3, KDR, TIMP3, AKT1, ITGAV, RAC1, PRKACA, HRAS, MAP2K1, MAP2K2, HGF, MMP2, WNT5A, PLAUR, RRAS2, MIR21, PRKCA, MMP9, RHOA, PIK3CA, SMO, CTTN, ITGA5, EZR, RAF1, SOS1, MET, TLR4, TP53, CD44, HBEGF, TLR2, SRC, PXN, TWIST1, PIK3R2, PIK3R1, CBL, THBS1, HIF1A, EGFR, CDC42, NRAS, PAK1, ERBB3, ERBB4, ERBB2, GPC3, FLNA, DROSHA, MAPK1, WNT1, MAPK3, TGFB2, FZD3, TGFB1, CAV1, PTCH1, STAT3, IGF2, FN1, PTPN11, BRAF, IGF1, MAPK14, ESR1, MTOR, PTK2, VEGFA, RPS6KB1, MDM2, PDCD4, CTNNB1, FAS, KRAS, NANOG, GRB2, HPSE, FGFR1

Table Ib. Gene ontology analysis of proteins in the metastatic process using the PANTHER database

Term	*P*-value	Genes
KEGG pathway		
hsa04151: PI3K-Akt signaling pathway	1.13E-40	CSF3, CSF1, IRS1, TNC, FGF1, FGF2, IGF1R, FGF4, CCND3, STK11, FGF7, CCND2, CCND1, PPP2R1A, MYC, AKT2, AKT3, MYB, KDR, AKT1, IL6R, PDGFRB, PDGFRA, MAP2K1, MAP2K2, HGF, TSC2, TSC1, PRKCA, PRLR, CCNE1, RAF1, TP53, EPHA2, CSF1R, PDGFB, PIK3R2, PIK3R1, FOXO3, BCL2L11, EIF4EBP1, MCL1, NGFR, INSR, FN1, IGF1, PTK2, NFKB1, IL2, IL4, COL1A1, IL3, IL6, CDK6, IL7, CDK4, GNB1, CDK2, BCL2, MDM2, GRB2, FGFR4, FGFR3, FGFR2, BCL2L1, FGFR1, ITGB1, YWHAE, GSK3B, CDKN1A, CDKN1B, FLT1, IFNA1, ITGB4, FLT4, ITGB3, IFNA2, PTEN, FASLG, BRCA1, PRL, PIK3CG, CASP9, ITGAV, RAC1, JAK2, HRAS, JAK1, HSP90AA1, CHUK, SYK, CREB1, PIK3CA, KIT, ITGA6, ITGA5, SOS1, MET, TLR4, TLR2, PRKAA1, THBS1, RELA, EGFR, INS, NRAS, SPP1, MAPK1, EIF4E, MAPK3, ANGPT2, BAD, IFNB1, EGF, NOS3, VEGFC, VEGFD, MTOR, VEGFA, KITLG, RPS6KB1, IL2RA, KRAS, TEK
hsa05215: Prostate cancer	4.11E-40	RB1, GSK3B, CDKN1A, CDKN1B, PTEN, PIK3CG, IGF1R, CASP9, CCND1, AKT2, AKT3, AKT1, EP300, HRAS, PDGFRB, PDGFRA, MAP2K1, HSP90AA1, MAP2K2, CHUK, AR, CREB1, PIK3CA, CCNE1, RAF1, SOS1, TP53, LEF1, PDGFB, TGFA, PIK3R2, KLK3, PIK3R1, FOXO1, EGFR, RELA, INS, NRAS, ERBB2, E2F1, MAPK1, E2F3, MAPK3, TCF7L2, CREBBP, SRD5A2, EGF, BAD, BRAF, IGF1, MTOR, NFKB1, NFKBIA, CDK2, BCL2, MDM2, CTNNB1, KRAS, GRB2, FGFR2, FGFR1

We obtained as many as 7356 proteins, and we correlated and selected the top 1000 proteins with the highest relevance scores. Then, we analyzed the gene ontology using the DAVID v6.8 database with three categories, namely biological process, cellular component, and molecular function. The extracellular matrix (ECM) composition and ECM compounds play key roles in the interaction between tumor cells and the microenvironment in the formation of the premetastatic niche (Paolillo and Schinelli, 2019). Therefore, we selected the proteins in the component of the extracellular matrix. These results confirmed that 55 proteins located in the extracellular matrix contributed to the metastatic process. Furthermore, KEGG pathway enrichment showed that several proteins participated in the regulation of pathways in cancer, microRNAs in cancer, proteoglycans in cancer, the PI3K-Akt signaling pathway, and prostate cancer (Table I). Thus, further analysis of the target prediction of jatropha seed meal compounds was needed.

The abovementioned signaling pathways regulate some important processes in cancer progression. The phosphoinositide 3-kinase (PI3K-Akt) signaling pathway controls cell proliferation, migration, growth, apoptosis, and other cancer processes (Hu *et al*., 2018). This pathway also encourages epithelial-mesenchymal transition (EMT) by regulating the expression of SNAI1, a transcription factor that stimulates EMT, leading to cancer invasion and migration. Inhibition of the PI3K-Akt pathway inhibits invasion, migration, and EMT of A549 lung cancer (Wang *et al*., 2018). Activation of the PI3K-Akt signaling pathway was shown to promote cell cycle progression in cervical cancer (Hu *et al*., 2018). Another study by Wang *et al*. (2018) showed that activation of the PI3K-Akt pathway facilitates cell growth and metastasis in non-small-cell lung cancer.

Predicted targets of jatropha seed meal compounds

Oskoueian *et al*. (2011) isolated various active compounds from jatropha seed meal. High-performance liquid chromatography analysis of methanolic extract showed the presence of daidzein, myricetin, gallic acid, and rutin. In addition, isoamericanol A was isolated from residues of expeller-pressed *Jatropha curcas* seeds (Katagi *et al*., 2016). To explore the predicted targets of jatropha seed meal compounds, we performed a SwissTargetPrediction database search, and each compound showed 100 target proteins (Figure 1A). In addition, Venn diagram analysis was used to determine the total predicted protein targets of jatropha seed compounds without duplication, resulting in a total of 307 proteins (Figure 1B).

A

No	Compound	Amount
1	Isoamericanol A	100
2	Daidzein	100
3	Myricetin	100
4	Gallic acid	100
5	Rutin	100
Total		307

B

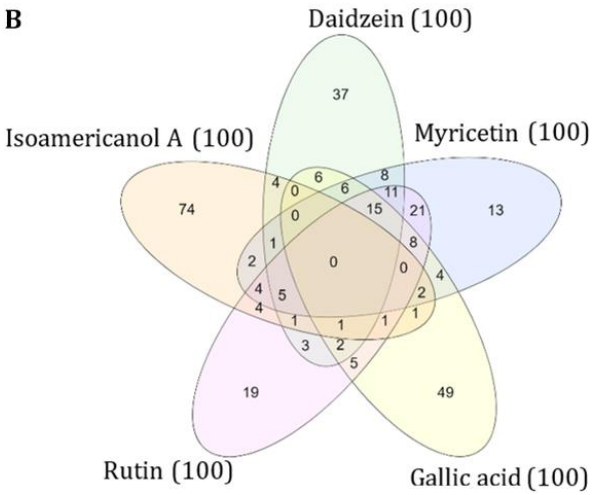


Figure 1. Target prediction for jatropha seed meal in the metastatic process (A) The predicted targets obtained from SwissTargetPrediction (B) Venn diagram of predicted targets of jatropha seed meal compounds resulting in 307 potential targets.

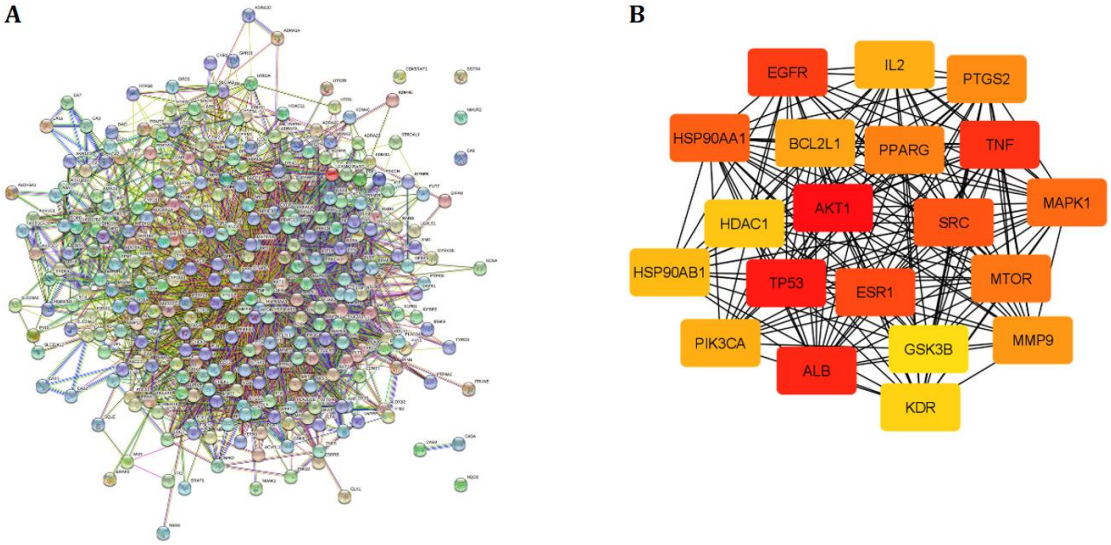


Figure 2. (A) Protein–protein interaction network of potential target proteins of jatropha seed meal compounds analyzed by STRING and (B) Top 20 hub genes based on highest degree score analyzed using Cytoscape.

PPI network analysis and hub gene selection

A total of 307 proteins were constructed in the PPI network, consisting of 302 nodes and 3137 edges, with an average node degree of 20.8 and a PPI enrichment *P*-value of <1.0e-16 (Figure 2A). We then employed the cytoHubba plugin to determine the 20 proteins with the highest degree scores and selected them as hub genes, including AKT1, TP53, ALB, TNF, EGFR, ESR1, SRC, HSP90AA1, MAPK1, MTOR, PPARG, PTGS2, MMP9. BCL2L1, PIK3CA, IL2, HSP90AB1, HDAC1, KDR, GSK3B (Table II). The hub genes are mainly involved in cancer signaling (AKT1, TP53, EGFR, HSP90AA1, MAPK1, MTOR, PPARG, PTGS2, MMP9, BCL2L1, PIK3CA, HDAC1, GSK3B).

We then compared the set of hub genes with that of extracellular matrix proteins to obtain a subset of predicted proteins in the jatropha seed meal compound. A Venn diagram generated two potential proteins, specifically MMP9 and HSP90AA1 (Figure 3).

Table II. Top 20 hub genes ranked by degree score

No	Gene symbol	Full name	Score
1	AKT1	AKT Serine/Threonine Kinase 1	270
2	TP53	Tumor Protein P53	254
3	ALB	Albumin	252
4	TNF	Tumor Necrosis Factor	242
5	EGFR	Epidermal Growth Factor Receptor	212
6	ESR1	Estrogen Receptor 1	206
7	SRC	SRC Proto-Oncogene, Nonreceptor Tyrosine Kinase	204
8	HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1	202
9	MAPK1	Mitogen-Activated Protein Kinase 1	178
10	MTOR	Mechanistic Target of Rapamycin Kinase	172
11	PPARG	Peroxisome Proliferator Activated Receptor Gamma	146
12	PTGS2	Prostaglandin-Endoperoxide Synthase 2	140
13	MMP9	Matrix Metalloproteinase 9	138
14	BCL2L1	BCL2 Like 1	114
15	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic	112
15	IL2	Interleukin 2	112
17	HSP90AB1	Heat Shock Protein 90 Alpha Family Class B Member 1	110
18	HDAC1	Histone Deacetylase 10	106
19	KDR	Kinase Insert Domain Receptor	104
20	GSK3B	Glycogen Synthase Kinase 3 Beta	100

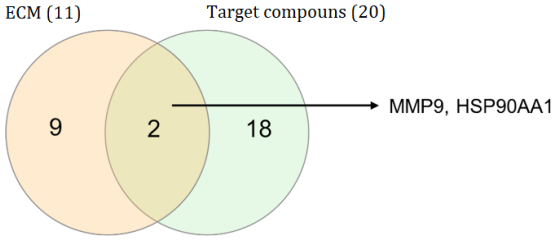


Figure 3. A Venn diagram of extracellular matrix proteins and hub genes.

MMP9 has been known for its biological functions and is commonly found in relation to cancer processes including invasion, metastasis, and angiogenesis (Huang, 2018). Moreover, HSP90AA1 was identified as a cytosolic protein essential for limiting membrane stress and maintaining protein homeostasis (Li *et al*., 2019). Therefore, we further investigated the potential of jatropha seed meal compounds as biomarkers of cancer development in comparison with that of MMP9.

Survival rate and expression analysis of the potential target protein

A survival analysis was performed to determine the potential of the target protein to affect the survival of patients with cancer. Using the GEPIA database, our evaluation revealed that mRNA expression of MMP9 was higher in tumor tissues than in normal tissues in all cancer types (COAD, KIRC, LUAD, and LIHC) (Figure 4A). The Kaplan–Meier plot indicates that patients with cancer and high expression of MMP9 in the kidney (p = 0.016) and liver (p = 0.0054) had significantly decreased overall survival than those with low expression of the same. Moreover, patients with low expression of MMP9 in colon (p = 0.96) and lung (p = 0.35) cancer had better overall survival than those with high levels of MMP9, but the difference was not significant (Figure 4B). We inferred those patients with higher MMP9 expression had worse overall survival than those with lower expression of the same. MMP9 overexpression has been observed in different malignant tumors and identified as a biomarker in different types of cancer (Huang, 2018).

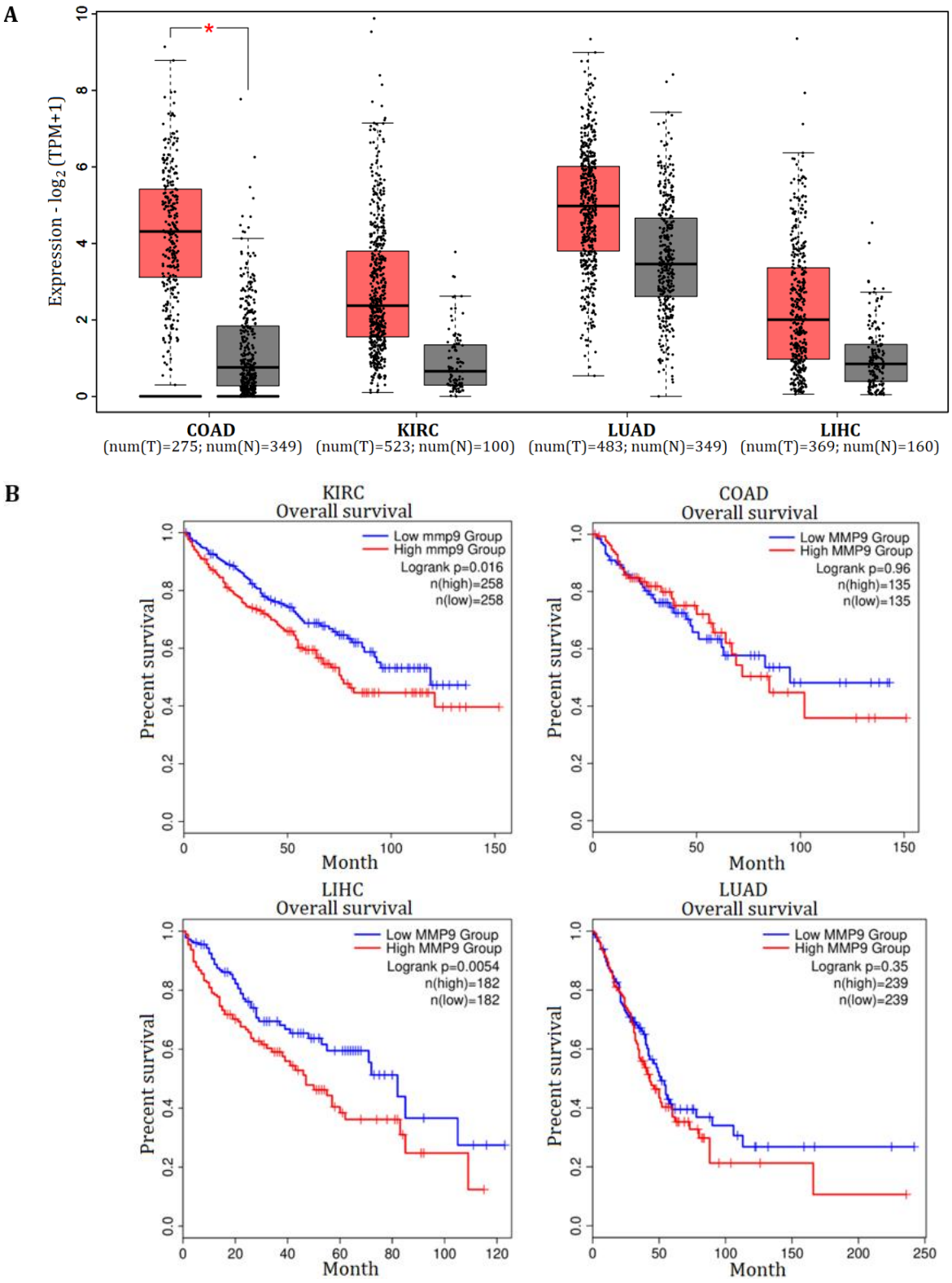


Figure 4. Survival analysis of MMP9 in several cancer datasets from the GEPIA database. (A) Comparison of the expression profile of MMP9 in tumor and normal tissues (B) Correlation of MMP9 expression to the overall survival of patients with cancer.

These phenomena suggest a crucial role of MMP9 in antimetastatic targeting of the jatropha seed meal compound.

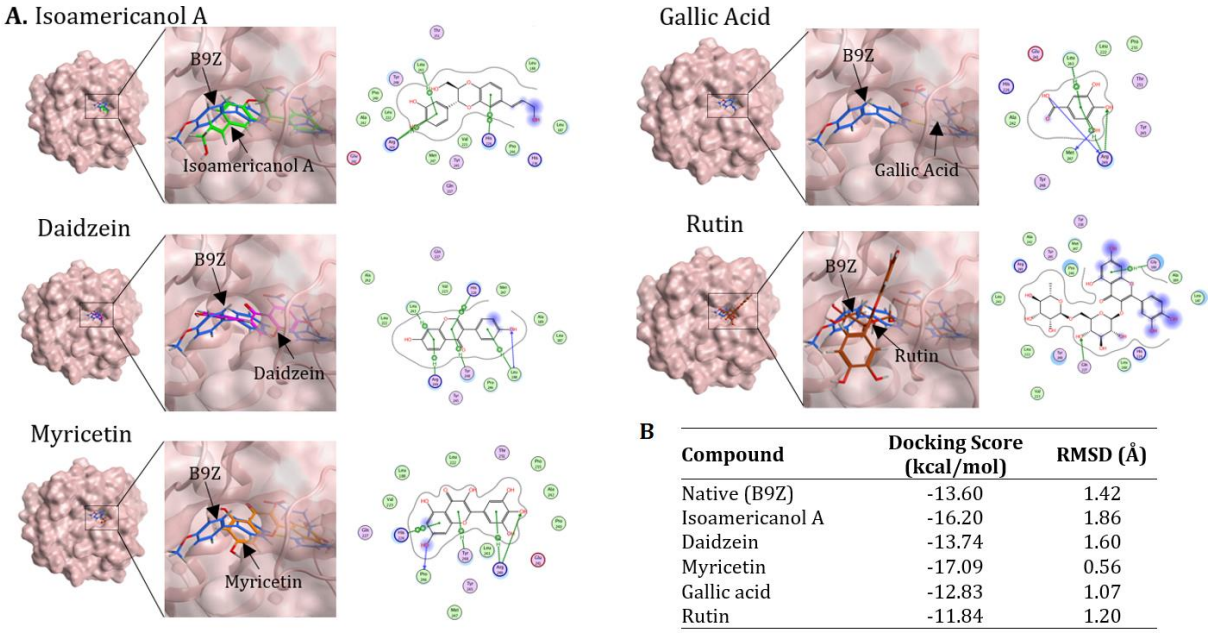
Potential of jatropha seed meal as an antimetastatic agent

Each part of the jatropha plant contains different compounds that are the sources of its usefulness in the health sector. Diterpenes are the main component of *Jatropha* and exert a variety of pharmacological effects (Abdelgadir and Van Staden, 2013). Inhibition of proteins that contribute to metastasis can be used as a molecular antimetastatic mechanism. In the present study, we predicted the ability of the compound present in jatropha seed meal to inhibit metastatic proteins. We performed molecular docking using MOE software and investigate several compounds known to have pharmacological activity to predict the antimetastatic activity of *Jatropha* seed meal components. The target protein used was MMP9 and the native ligand was B9Z (2-{S})-2-[2-[4-(4 methoxyphenyl) phenyl] sulfanylphenyl] pentanedioic acid). MMP9, a protease of the matrix metalloproteinases (MMPs) family that plays a crucial role in cancer metastasis, is responsible for degradation of the ECM and is involved in several signaling pathways. Isoamericanol A, daidzein, and myricetin showed lower docking scores than native ligands, with binding energy interaction values of −16.20, −13.73, and −17.09 kcal/mol, respectively (Figure 5B). These results demonstrate that MMP9 has a higher binding affinity for isoamericanol A, daidzein, and myricetin than native ligands (Figure 5B). Meanwhile, gallic acid and rutin exhibited higher docking scores than native ligands, which means their binding affinity is lower than that of native ligands, with ΔG values of −12.83 and −11.84 kcal/mol, respectively. This result can be attributed to Arg 249 and His 226, amino acid residues positioned between docking compounds except for gallic acid and rutin (Figure 5A). Meanwhile, gallic acid showed other interactions at the Arg 249 residue, which potentially contributed to its binding activity, whereas rutin had no interaction with those amino acids, resulting in a lower binding affinity than that of the native ligand. All these data indicate the potential inhibitory activity of compounds contained in jatropha seed meal against MMP9 and their development as antimetastatic agents.

This study aimed to elucidate the potential of jatropha seed meal compounds as chemopreventive agents by targeting metastatic processes using molecular docking and bioinformatics approaches. Metastasis is a complex process and involves several cellular and molecular events by which cancer cells develop the ability to migrate to and invade other organs (Liao *et al*., 2012). In this study, we analyzed the regulatory proteins of the metastatic process (Table 1) and aligned these proteins with potential targets of jatropha seed compounds (Figure 1). Furthermore, we performed PPI network analysis and hub gene selection and found two predicted protein targets of jatropha seed compounds, i.e., MMP9 and HSP90AA1 (Figure 2 and 3). HSP90AA1 is a protein known to maintain cell homeostasis; therefore, we focused on MMP9. In further analysis, we showed that MMP9 plays an important role in tumor progression, and high expression of this protein lowers the survival rate of patients with cancer (Figure 4 and 5). High MMP expression is correlated with poor cancer prognosis and implicated in almost all steps of metastasis (Paolillo and Schinelli, 2019). Among these families, MMP9 (gelatinase B) is highly associated with tumor dissemination and invasion via degradation of type IV collagen and gelatin substrates (Webb *et al*., 2017).

Phorbol esters which are considered toxic compounds in jatropha oil and seeds cause skin irritation and promote tumor formation by stimulating protein kinase C. Among the phorbol esters present in jatropha, 12-Deoxy-16-hydroxyphorbol has demonstrated the tumor-promoting activity (Li *et al*., 2010; Abdelgadir and Van Staden, 2013; Fujiki *et al*., 2017). In the process of oil extraction from jatropha seeds, 70%–75% of phorbol esters will be sequestered in jatropha oil, and 25%–30% will be bound to byproducts of the extraction process, namely, jatropha seed meal (Gogoi *et al*., 2014). Phorbol esters must be removed from jatropha seed meal to increase its beneficial properties (Abou-Arab *et al*., 2019).

Some phenolics, flavonoids, and iso-flavonoids are found in jatropha seed meal extract. Methanol extract of jatropha seed meal exhibited the major phenolic compound gallic acid (581 μg/g). Other compounds found in jatropha seed meal extract included rutin (48 μg/g), myricetin (199 μg/g), and daidzein (298 μg/g) (Oskoueian *et al*., 2011). Using an *in silico* approach, the current study revealed that compounds in jatropha seed, i.e., isomericanol A, daidzein myricetin, gallic acid, and rutin, have the potential to bind to MMP9 with a comparable if not higher affinity than that of the native ligand, B9Z (Figure 5).



B

Compound	Docking Score (kcal/mol)	RMSD (Å)
Native (B9Z)	-13.60	1.42
Isoamericanol A	-16.20	1.86
Daidzein	-13.74	1.60
Myricetin	-17.09	0.56
Gallic acid	-12.83	1.07
Rutin	-11.84	1.20

Figure 5. Molecular docking simulation of the molecular interaction of *Jatropha curcas* with MMP9. (A) The crystal structure of MMP9 and the docking position of *Jatropha curcas* compounds, as well as the corresponding amino acids, are shown in the right panel. The position of native ligand binding is shown. (B) Docking score and RMSD of *Jatropha curcas* compound against MMP9.

It has been reported that the methanol extract of jatropha seed meal inhibited the growth and proliferation of MCF-7 breast cancer cells with an IC_{50} value of 27.5 µg/mL and HeLa cervical cancer cells with an IC_{50} of 56.4 µg/mL. Additional findings showed that isoamericanol A inhibited the growth of cancer cells such as MCF-7 and MDA-MB 231 breast cancer cells, Huh-7 liver cancer cells, and HeLa cervical cancer cells, starting at a dose of 25 µg/ml. Isoamericanol A induced cell cycle arrest in the G2/M phase in MCF-7 cells. In addition, it decreased the expression of cyclin B1, cyclin B2, and cyclin-dependent kinase 1 (CDK1) (Katagi *et al*., 2016). Rutin, a flavonoid, induces G2/M arrest and cell apoptosis toward human neuroblastoma cells LAN-5 in a dose-dependent manner. It also reduces Bcl2 expression and the Bcl2/Bax ratio (Chen *et al*., 2013).

Myricetin decreased MMP9 protein expression in MDA-MB-231br breast cancer cells (Ci *et al*., 2018). In addition, it promoted apoptosis by inhibiting Bcl-2, an apoptotic suppression gene, and activating the expression of Bax and caspase-3, proteins that stimulate the apoptotic process in the T24 bladder cancer cell line (Sun *et al*., 2012). Another study confirmed that myricetin inhibited the invasion and migration of the A549 lung cancer cell line through inactivation of the ERK1 and ERK2 pathways (Shih *et al*., 2009). Meanwhile, daidzein reduced the expression of MMP9 protein in DU-145 prostate cancer cells (Leiva *et al*., 2015). MMP9 is a group of gelatinases that play an essential function in metastasis, invasion, migration, and angiogenesis of cancer cells (Huang, 2018; Jiang *et al*., 2019). The ability of compounds in jatropha seed meal to inhibit invasion and migration in highly metastatic cancer cells, such as MDA-MB-231 breast cancer cells and A549 lung cancer cells, indicates that jatropha seed meal has the potential to inhibit metastasis through reduced MMP9 expression. In this case, we used MMP9 as a target of molecular docking. Moreover, gallic acid inhibited MCF-7 cell viability at concentrations below 10 µM and induced cell cycle arrest at concentrations above 50 µM (Chen *et al*., 2016). Another study showed that gallic acid suppressed cell migration and MMP9 and MMP2 activities in U-2 OS bone cancer cells at concentrations of 20–40 µM. In addition, gallic acid downregulated ERK1/2, which plays a role in the ERK signaling pathway in triggering the invasion and metastatic cancer cells (Liao *et al*., 2012). Gallic acid reduced the migration of AGS gastric cancer cells by inhibiting the activity of NF-κB, a regulator of MMP2 and MMP9 gene

expression in the metastatic by degrading the ECM (Ho *et al*., 2010).

Our findings showed that jatropha seed meal compounds, especially isoamericanol A, daidzein, and myricetin, have a higher binding interaction affinity than the native ligand (Figure 5). In addition, in vitro experiments on MDA-MB-231 cells showed that myricetin exhibited migration, invasion, and adhesion at concentrations of 5 and 10 μM. At the same concentration, myricetin suppressed the expression of MMP2 and MMP9 (Ci *et al*., 2018). Meanwhile, daidzein decreased TNF-α-induced MMP9 activity on HaCaT keratinocytes and reduced DU-145 prostate cancer cell invasion by 59.8% (Leiva *et al*., 2015). These findings revealed that jatropha seed meal compounds are possibly effective chemopreventive agents against cancer cells through their cytotoxic activity, inhibition of cell migration and several signaling pathways, and reduced expression of MMP2 and MMP9. These effects lead to the inhibition of cancer metastasis.

Although jatropha seed meal and its compounds exhibit antimetastatic activity, their use as a supportive agent is still limited. Problems in the processing, use, and distinctive smell of natural ingredient extracts are the main obstacles. Jatropha seed meal must be prepared in nutraceutical dosage forms to increase its acceptability and stability. Nutraceuticals are products that contain active ingredients and provide health benefits. Nutraceuticals have broad applications as health products, some of which employ the capsule dosage form. Capsules avoid the unpleasant taste and odor of the drug and are easier to swallow than tablets (Hoag, 2017). The capsule shell can be modified to target drug release at specific target organs (Gullapalli and Mazzitelli, 2017). The production of jatropha seed oil can be performed continuously to generate jatropha seed meal as an economically important waste product. Jatropha seed meal is easily processed into a pharmaceutical dosage form with minimal cost. Based on these advantages, jatropha seed meal can be formulated into capsules with antimetastatic efficacy as a renewable innovation that can increase the economic value and usefulness of the jatropha plant.

CONCLUSION

Jatropha seed meal contains bioactive compounds, including isoamericanol A, myricetin, daidzein, gallic acid, and rutin, that are conducive to its anticancer effectiveness. These compounds target the proteins involved in cancer metastasis located in the extracellular matrix, specifically MMP9. We observed that the compounds in jatropha seed meal possessed binding affinity for MMP9, with a comparable docking score to that of the native ligand. Thus, the present study indicated that jatropha seed meal is a potential candidate as an antimetastatic agent. It can be developed into pharmaceutical dosage forms to increase its acceptability and economic value. Further *in vitro* and *in vivo* experiments are needed to validate this finding.

ACKNOWLEDGEMENT

We thank Prof. Dr. apt. Edy Meiyanto, M.Si. (The Head of Laboratory of Macromolecular Engineering, Department of Pharmaceutical Chemistry and the Head of Cancer Chemoprevention Research Center, Faculty of Pharmacy Universitas Gadjah Mada) for giving insightful comments and supports on our research.

REFERENCES

Abdelgadir, H. A., & Van Staden, J. (2013). Ethnobotany, ethnopharmacology and toxicity of Jatropha curcas L. (Euphorbiaceae): A review. *South African Journal of Botany*, *88*, 204–218. https://doi.org/10.1016/j.sajb.2013.07.021

Abou-Arab, A. A., Mahmoud, M. H., Ahmed, D. M. M., & Abu-Salem, F. M. (2019). Comparative study between chemical, physical and enzymatic methods for Jatropha curcas kernel meal phorbol ester detoxification. *Heliyon*, *5*(5), e01689. https://doi.org/10.1016/j.heliyon.2019.e01689

Alonso-Castro, A.J., Villarreal, M.L., Salazar-Olivo, L.A., Gomez-Sanchez, M., Dominguez, F., Garcia-Carranca, A., 2011. Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. Journal of Ethnopharmacology 133, 945–972.

Asuk, A. A., Agiang, M. A., Dasofunjo, K., & Willie, A. J. (2015). The biomedical significance of the phytochemical, proximate and mineral compositions of the leaf, stem bark and root of Jatropha curcas. *Asian Pacific Journal of Tropical Biomedicine*, *5*(8), 650–657. https://doi.org/10.1016/j.apjtb.2015.05.015

Chen, H., Miao, Q., Geng, M., Liu, J., Hu, Y., Tian, L., Pan, J., & Yang, Y. (2013). Anti-tumor effect

of rutin on human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. *The Scientific World Journal*, *2013*, 1–8. https://doi.org/10.1155/2013/269165

Chen, Y.-J., Lin, K.-N., Jhang, L.-M., Huang, C.-H., Lee, Y.-C., & Chang, L.-S. (2016). Gallic acid abolishes the EGFR/Src/Akt/Erk-mediated expression of matrix metalloproteinase-9 in MCF-7 breast cancer cells. *Chemico-Biological Interactions*, *252*, 131–140. https://doi.org/10.1016/j.cbi.2016.04.025

Ci, Y., Zhang, Y., Liu, Y., Lu, S., Cao, J., Li, H., Zhang, J., Huang, Z., Zhu, X., Gao, J., & Han, M. (2018). Myricetin suppresses breast cancer metastasis through down-regulating the activity of matrix metalloproteinase (MMP)-2/9. *Phytotherapy Research*, *32*(7), 1373–1381. https://doi.org/10.1002/ptr.6071

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, *47*(W1), W357–W364. https://doi.org/10.1093/nar/gkz382

Eble, J. A., & Niland, S. (2019). The extracellular matrix in tumor progression and metastasis. *Clinical and Experimental Metastasis*, *36*(3), 171–198. https://doi.org/10.1007/s10585-019-09966-1

Fujiki, H., Suttajit, M., Rawangkan, A., Iida, K., Limtrakul, P., Umsumarng, S., & Suganuma, M. (2017). Phorbol esters in seed oil of Jatropha curcas L. (saboodam in Thai) and their association with cancer prevention: From the initial investigation to the present topics. *Journal of Cancer Research and Clinical Oncology*, *143*(8), 1359–1369. https://doi.org/10.1007/s00432-017-2341-6

Gogoi, R., Niyogi, U. K., & Tyagi, A. K. (2014). Reduction of phorbol ester content in jatropha cake using high energy gamma radiation. *Journal of Radiation Research and Applied Sciences*, *7*(3), 305–309. https://doi.org/10.1016/j.jrras.2014.04.002

Gullapalli, R. P., & Mazzitelli, C. L. (2017). Gelatin and non-gelatin capsule dosage forms. *Journal of Pharmaceutical Sciences*, *106*(6), 1453–1465. https://doi.org/10.1016/j.xphs.2017.02.006

Heberle, H., Meirelles, G. V., da Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, *16*(1), 169. https://doi.org/10.1186/s12859-015-0611-3

Hermawan, A., Khumaira, A., Ikawati, M., Putri, H., Jenie, R. I., Angraini, S. M., & Muflikhasari, H. A. (2021). Identification of key genes of hesperidin in inhibition of breast cancer stem cells by functional network analysis. *Computational Biology and Chemistry*, *90*, 107427. https://doi.org/10.1016/j.compbiolchem.2020.107427

Hermawan, A., Putri, H., & Utomo, R. Y. (2020). Comprehensive bioinformatics study reveals targets and molecular mechanism of hesperetin in overcoming breast cancer chemoresistance. *Molecular Diversity*, *24*(4), 933–947. https://doi.org/10.1007/s11030-019-10003-2

Ho, H.-H., Chang, C.-S., Ho, W.-C., Liao, S.-Y., Wu, C.-H., & Wang, C.-J. (2010). Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-κB activity and downregulation of PI3K/AKT/small GTPase signals. *Food and Chemical Toxicology*, *48*(8–9), 2508–2516. https://doi.org/10.1016/j.fct.2010.06.024

Hoag, S. W. (2017). Capsules Dosage Form. In *Developing Solid Oral Dosage Forms* (pp. 723–747). Elsevier. https://doi.org/10.1016/B978-0-12-802447-8.00027-3

Hu, R., Wang, M. Q., Niu, W. B., Wang, Y. J., Liu, Y. Y., Liu, L. Y., Wang, M., Zhong, J., You, H. Y., Wu, X. H., Deng, N., Lu, L., & Wei, L. B. (2018). SKA3 promotes cell proliferation and migration in cervical cancer by activating the PI3K/Akt signaling pathway. *Cancer Cell International*, *18*(1), 183. https://doi.org/10.1186/s12935-018-0670-4

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, *4*(1), 44–57. https://doi.org/10.1038/nprot.2008.211

Huang, H. (2018). Matrix metalloproteinase-9 (MMP9) as a cancer biomarker and MMP9 biosensors: Recent advances. *Sensors*,

18(10), 3249. https://doi.org/10.3390/s18103249

Huang, S.-L., Wang, W.-H., Zhong, X.-Y., Lin, C.-T., Lin, W.-S., Chang, M.-Y., & Lin, Y.-S. (2020). Antioxidant properties of Jatropha curcas L. seed shell and kernel extracts. *Applied Sciences*, *10*(9), 3279. https://doi.org/10.3390/app10093279

Jaramillo-Quintero, L. P., Contis Montes de Oca, A., Romero Rojas, A., Rojas-Hernández, S., Campos-Rodríguez, R., & Martínez-Ayala, A. L. (2015). Cytotoxic effect of the immunotoxin constructed of the ribosome-inactivating protein curcin and the monoclonal antibody against Her2 receptor on tumor cells. *Bioscience, Biotechnology, and Biochemistry*, *79*(6), 896–906. https://doi.org/10.1080/09168451.2015.1006572

Jiang, M., Zhu, M., Wang, L., & Yu, S. (2019). Anti-tumor effects and associated molecular mechanisms of myricetin. *Biomedicine and Pharmacotherapy*, *120*, 109506. https://doi.org/10.1016/j.biopha.2019.109506

Katagi, A., Sui, L., Kamitori, K., Suzuki, T., Katayama, T., Hossain, A., Noguchi, C., Dong, Y., Yamaguchi, F., & Tokuda, M. (2016). Inhibitory effect of isoamericanol A from Jatropha curcas seeds on the growth of MCF-7 human breast cancer cell line by G2/M cell cycle arrest. *Heliyon*, *2*(1), e00055. https://doi.org/10.1016/j.heliyon.2015.e00055

Katagi, A., Sui, L., Kamitori, K., Suzuki, T., Katayama, T., Hossain, A., Noguchi, C., Dong, Y., Yamaguchi, F., & Tokuda, M. (2016b). Inhibitory effect of isoamericanol A from Jatropha curcas seeds on the growth of MCF-7 human breast cancer cell line by G2/M cell cycle arrest. *Heliyon*, *2*(1), e00055. https://doi.org/10.1016/j.heliyon.2015.e00055

Katagi, A., Sui, L., Kamitori, K., Suzuki, T., Katayama, T., Hossain, A., Noguchi, C., Dong, Y., Yamaguchi, F., & Tokuda, M. (2017). High anticancer properties of defatted *Jatropha curcus* seed residue and its active compound, Isoamericanol A. *Natural Product Communications*, *12*(12), 1934578X1701201. https://doi.org/10.1177/1934578X1701201217

Leiva, B., Carrasco, I., Montenegro, I., Gaete, L., Lemus, I., Tchernitchin, A., Bustamante, R., Párraga, M., & Villena, J. (2015). *Equol and daidzein decrease migration, invasion and matrix metalloproteinase (MMPs) gene expression in prostate cancer cell lines, DU-145 and PC-3*. 13.

Li, C.-Y., Devappa, R. K., Liu, J.-X., Lv, J.-M., Makkar, H. P. S., & Becker, K. (2010). Toxicity of Jatropha curcas phorbol esters in mice. *Food and Chemical Toxicology*, *48*(2), 620–625. https://doi.org/10.1016/j.fct.2009.11.042

Li, P., Wang, J., Zou, Y., Sun, Z., Zhang, M., Geng, Z., Xu, W., & Wang, D. (2019). Interaction of Hsp90AA1 with phospholipids stabilizes membranes under stress conditions. *Biochimica et Biophysica Acta - Biomembranes*, *1861*(2), 457–465. https://doi.org/10.1016/j.bbamem.2018.11.009

Liang, M.M., Teo, S. Y., Gudi, M., Lim, S. H., & Win, T. (2019). Breast microcalcifications as the only imaging manifestation of metastatic serous peritoneal adenocarcinoma in the breast. *Journal of Radiology Case Reports*, *13*(10), 11–20. https://doi.org/10.3941/jrcr.v13i10.3769

Liao, C.-L., Lai, K.-C., Huang, A.-C., Yang, J.-S., Lin, J.-J., Wu, S.-H., Gibson Wood, W., Lin, J.-G., & Chung, J.-G. (2012). Gallic acid inhibits migration and invasion in human osteosarcoma U-2 OS cells through suppressing the matrix metalloproteinase-2/-9, protein kinase B (PKB) and PKC signaling pathways. *Food and Chemical Toxicology*, *50*(5), 1734–1740. https://doi.org/10.1016/j.fct.2012.02.033

Liu, J.-Q., Yang, Y.-F., Wang, C.-F., Li, Y., & Qiu, M.-H. (2012). Three new diterpenes from Jatropha curcas. *Tetrahedron*, *68*(4), 972–976. https://doi.org/10.1016/j.tet.2011.12.006

Luo, M.J., Liu, W.X., Yang, X.Y., Xu, Y., Yan, F., Huang, P., Chen, F., 2007. Cloning, expression and antitumor activity of recombinant protein of curcin. Russian Journal of Plant Physiology 54, 202–206.

Marrufo-Estrada, D. M., Segura-Campos, M. R., Chel-Guerrero, L. A., & Betancur-Ancona, D. A. (2013). Defatted Jatropha curcas flour and protein isolate as materials for protein hydrolysates with biological activity. *Food Chemistry*, *138*(1), 77–83. https://doi.org/10.1016/j.foodchem.2012.09.033

Montes, J. M., & Melchinger, A. E. (2016). Domestication and Breeding of Jatropha curcas L. *Trends in Plant Science*, *21*(12), 1045–1057. https://doi.org/10.1016/j.tplants.2016.08.008

Muangman, S., Thippornwong, M., & Tohtong, R. (2005). Anti-metastatic effects of curcusone B, a diterpene from Jatropha curcas. *In Vivo*, 19(1), 265–268.

Oskoueian, E., Abdullah, N., Ahmad, S., Saad, W. Z., Omar, A. R., & Ho, Y. W. (2011). Bioactive compounds and biological activities of Jatropha curcas L. kernel meal extract. *International Journal of Molecular Sciences*, *12*(9), 5955–5970. https://doi.org/10.3390/ijms12095955

Pandey, V. C., Singh, K., Singh, J. S., Kumar, A., Singh, B., & Singh, R. P. (2012). Jatropha curcas: A potential biofuel plant for sustainable environmental development. *Renewable and Sustainable Energy Reviews*, *16*(5), 2870–2883. https://doi.org/10.1016/j.rser.2012.02.004

Paolillom, M., & Schinelli, S. (2019). Extracellular matrix alterations in metastatic processes. *International Journal of Molecular Sciences*, *20*(19), 4947. https://doi.org/10.3390/ijms20194947

Robinson, D. R., Wu, Y.-M., Lonigro, R. J., Vats, P., Cobain, E., Everett, J., et al (2017). Integrative clinical genomics of metastatic cancer. *Nature*, *548*(7667), 297–303. https://doi.org/10.1038/nature23306

Safran, M., Rosen, N., Twik, M., BarShir, R., Stein, T. I., Dahary, D., Fishilevich, S., & Lancet, D. (2021). The GeneCards Suite. In I. Abugessaisa & T. Kasukawa (Eds.), *Practical Guide to Life Science Databases* (pp. 27–56). Springer Singapore. https://doi.org/10.1007/978-981-16-5812-9_2

Sharma, A. K., Gangwar, M., Tilak, R., Nath, G., Sinha, A. S. K., Tripathi, Y. B., & Kumar, D. (2012). Comparative in vitro antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of Jatropha curcas linn. *Pharmacognosy Journal*, *4*(30), 34–40. https://doi.org/10.5530/pj.2012.30.7

Shih, Y.-W., Wu, P.-F., Lee, Y.-C., Shi, M.-D., & Chiang, T.-A. (2009). Myricetin suppresses invasion and migration of human lung adenocarcinoma A549 cells: Possible mediation by blocking the ERK signaling pathway. *Journal of Agricultural and Food Chemistry*, *57*(9), 3490–3499. https://doi.org/10.1021/jf900124r

Sun, F., Zheng, X. Y., Ye, J., Wu, T. T., Wang, J. li, & Chen, W. (2012). Potential anticancer activity of myricetin in human T24 bladder cancer cells both in vitro and in vivo. *Nutrition and Cancer*, *64*(4), 599–606. https://doi.org/10.1080/01635581.2012.665564

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Research*, *49*(D1), D605–D612. https://doi.org/10.1093/nar/gkaa1074

Tang, Z., Li, C., Kang, B., Gao, G., Li, C., & Zhang, Z. (2017). GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Research*, *45*(W1), W98–W102. https://doi.org/10.1093/nar/gkx247

Wang, S., Yan, Y., Cheng, Z., Hu, Y., & Liu, T. (2018). Sotetsuflavone suppresses invasion and metastasis in non-small-cell lung cancer A549 cells by reversing EMT via the TNF-α/NF-κB and PI3K/AKT signaling pathway. *Cell Death Discovery*, *4*(1), 26. https://doi.org/10.1038/s41420-018-0026-9

Webb, A. H., Gao, B. T., Goldsmith, Z. K., Irvine, A. S., Saleh, N., Lee, R. P., et al. (2017). Inhibition of MMP2 and MMP9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. *BMC Cancer*, *17*(1), 434. https://doi.org/10.1186/s12885-017-3418-y

Yao, L., Han, C., Chen, G., Song, X., Chang, Y., & Zang, W. (2012). A new asymmetric diamide from the seed cake of Jatropha curcas L. *Fitoterapia*, *83*(8), 1318–1321. https://doi.org/10.1016/j.fitote.2012.04.001