Science and Technology Indonesia
*e*-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 9, No. 2, April 2024

Research Paper

# Network Pharmacology and Component Analysis Integrated Study to Uncovers the Molecular Mechanisms of *Lansium parasiticum* Bark Extract in Colon Cancer Treatment

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**Abstract**
Side effects and risk of resistance are common consequences of colon cancer treatment based on chemotherapy. The medicinal plant originating in Indonesia, *Lansium parasiticum* bark extract (LPBE), has not been studied much. The purpose of this study is to identify the compounds present in LPBE and explain how the molecular mechanisms of the composite inhibit colon cancer cells. LC-MS/MS Liquid Chromatography Tandem Mass Spectrophotometry has been used to identify compounds in LPBE. The ADMET program is used to determine absorption profiles and bioavailability per oral. The tissue pharmacology approach uses Cytoscape 3.9.1, GeneCards, Disgenet, STRING 2.0.0, SRplot, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway to predict the molecular anti-cancer mechanisms of these compounds. In vitro validation using PyRx Autodock Vina 9.0 and Biovia Discovery Studio with BAX (PDB ID:2YXJ), BCL2 (P DB ID:2W3L) and STAT3 receptors (PDB ID:6NJS). A total of 17 active compounds were identified through LC-MS/MS. The moronic acid compound showed the highest levels of 14.29% followed by 4-Morpholineacetic Acid 12.2% and ursolic aldehyde 8.37%. Pharmacological network analysis showed that the compounder works on the EGFR tyrosine kinase resistance path by targeting the BCL2, BAX, STAT3 genes. The results of the in silico validation support the results of tissue pharmacology findings. Ursolic aldehyde, and Moronic acid showed a higher affinity to the three receptors. Therefore, *Lansium parasiticum* bark extract (LPBE) is recommended for further study as a candidate anti-cancer drug both in vitro and in vivo.

**Keywords**
BCL2, BAX, EGFR, Moronic Acid, STAT3

Received: 25 November 2023, Accepted: 14 February 2024
https://doi.org/10.26554/sti.2024.9.2.314-324

## 1. INTRODUCTION

Colorectal cancer (CRC) is a prevalent malignancy that has consistently ranked as the third most frequently occurring cancer in males and the second most prevalent in females globally during recent decades (Douaiher et al., 2017). Moreover, it is noteworthy that this particular illness ranks as the third most lethal form of cancer within the United States (Ionescu et al., 2023; Vega et al., 2015). The number of fatalities decreased from 49.190 in 2016 to 37.930 in 2020 (Xi and Xu, 2021). However, it is projected that the morbidity rate will see a tenfold increase by the year 2035 (Liu et al., 2015). Various indicators, including socio-economic characteristics such as the Human Development Index (HDI) score, life expectancy, dietary patterns, lifestyle choices, and geographical considerations all suggest a global rise in morbidity (Kuipers et al., 2015). The efficacy and adverse effects of conventional treatments for colon cancer, including chemotherapy, radiation therapy, and hormone therapy, are subject to certain limits. However, the availability of targeted therapy for colon cancer, which is capable of specifically targeting genetic abnormalities, remains limited (Sawicki et al., 2021). Currently, there remains a pressing need to continue the development of novel anti-cancer pharmaceuticals in order to address ongoing challenges in this field.

*Lansium parasiticum*, commonly known as *Lansium*, is a botanical species with medicinal properties that is distributed in several regions of Indonesia. Traditional medicine frequently employs this treatment modality for several ailments, including as parasitic infections, fever reduction, alleviation of gastrointestinal distress, and administration of anticancer agents. The prevailing belief within local populations is that the therapeutic properties of this fruit are mostly derived from its fruit and leaves, whereas other portions of the plant, such as the stem's skin, are seldom utilized as a fundamental component in medicinal preparations (Abdallah et al., 2022). Previous research has that the skin extract of *Lansium parasiticum* (LPBE) contains

flavonoid components (Fadhilah et al., 2021).

Pharmacological research proves that the plant has a wide range of bioactivities, including antimalarial, antifeed, anti-aging, wound healing, antioxidant, cytotoxic, analgesic, antibacterial, antimutagenic, insecticide, and larvasidal properties. The most commonly described activity is due to the presence of terpenoids and phenolics (Potipiranun et al., 2018; Goel et al., 2020; Fadhilah et al., 2021; Lubis et al., 2022).

Research data on the overall active content of the lancium parasitium strain is still lacking. Furthermore, the anti-cancer activity of the active compound in the stem of this plant has never been evaluated. On the other hand, previous research has not focused on the identification of molecular mechanisms, target genes, and pathways associated with substances that have the ability to prevent the growth of cancer cells in extracts of this plant's stem. Therefore, the novelty of this study is to reveal the metabolites of the compound, the anti-cancer activity of the composition, the molecular mechanisms, the target genes, and the potential pathways of the active compounds associated with the therapeutic effects of *Lansium parasiticum* skin extract (LPBE) in the management of colorectal cancer.In order to elucidate the development of disease from the perspective of systems biology, pharmacology, and biological tissue, the field of network pharmacology has emerged as a promising research methodology. Network pharmacology integrates the disciplines of pharmacology, molecular biology, and bioinformatics to discern the interconnections between pharmacologically active components, their associated targets, signaling pathways, and diseases within specific tissues (Berger and Iyengar, 2009).

The primary aim of this study was to conduct metabolite profiling of compounds utilizing the LCMS/MS technique, followed by an examination of the molecular mechanisms, target genes, and potential pathways associated with the therapeutic effects of *Lansium parasiticum* bark extract (LPBE) in the management of colon cancer. This investigation employed Network pharmacology and bioinformatics methodologies.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials and Instrumentation

The materials used in the experimental procedure included an Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS/MS) system (Waters,USA). The UPLC-MS/MS system was equipped with an Acquity C18 column, featuring a particle size of 1.8 µm and dimensions of 2.1 × 150 mm. The eluent components consisted of two solutions: Component A, comprising high-performance liquid chromatography grade water and formic acid sourced (Merck, Darmstadt, Germany), with a ratio of 99.9:0.1 (water to formic acid), and Component B, consisting of acetonitrile and formic acid (Merck, Darmstadt, Germany), also with a ratio of 99.9:0.1 (acetonitrile to formic acid). The solvent used was absolute methanol (Merck, Darmstadt, Germany). Analytical settings were managed using Mass Lynx version 4.1 software developed (Waters, USA). Compound identification was facilitated through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

The ADMET program was utilized to determine oral absorption profiles and bioavailability (https://admetmesh.scbdd.com/). A tissue pharmacology approach was adopted utilizing Cytoscape 3.9.1, GeneCards (https://www.genecards.org), Disgenet (https://www.disgenet.org), STRING 2.0.0 (https://string-db.org/), SRplot (https://www.bioinformatics.com.cn/en), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway to predict the molecular anti-cancer mechanisms of these compounds. In vitro validation was conducted using PyRx Autodock Vina 9.0 and Biovia Discovery Studio with receptors BAX (PDB ID:2YXJ), BCL2 (PDB ID:2W3L), and STAT3 (PDB ID:6NJS).

### 2.2 Methods

#### 2.2.1 Extract from the Roots of *Lansium parasicum* (LPBE) Preparation

The process of extracting *Lansium parasiticum* roots (LPBE) was performed utilizing Lansium parasiticum roots obtained under the reference number 067/566/102.20/2023 from a specific region in East Java, situated at an elevation of 400 meters. The region exhibited a mean temperature of 25 degrees Celsius and an average yearly precipitation of 125.49 mm. The powdered roots underwent extraction at a ratio of 1:10 using a solution of 70% ethanol and the Ultra Assisted Extraction (UAE) technique, conducted at a temperature of 40°C for a duration of 20 minutes. Following that, the ethanol extract was subjected to preparation for subsequent analysis through its placement in an oven that was adjusted to a temperature of 40°C for a duration of 5 hours.

#### 2.2.2 The LC-MS/MS Technique is Employed for Analysis

The LC-MS/MS analysis was performed utilizing Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) systems equipped with a Quadrupole Time-of-Flight (QToF) analyzer and employing positive Electrospray Ionization (ESI) as the ionization source. The experimental setup employed an Acquity C18 column with dimensions of 1.8 µm in particle size and 2.1 × 150 mm in column dimensions. The eluent utilized in this study was composed of two components. The first component, referred to as A, consisted of water of high-performance liquid chromatography (HPLC) grade, mixed with formic acid obtained from Merck, Darmstadt, Germany. The ratio of water to formic acid in component A was 99.9:0.1 [v/v]. The second component, referred to as B, consisted of acetonitrile obtained from Merck, Darmstadt, Germany, mixed with formic acid. The ratio of acetonitrile to formic acid in component B was also 99.9:0.1 [v/v]. The elution process employed a gradient system. The temperature of the source was adjusted to 100°C, while the desolvation temperature was set at 350°C. A 10 milligram (mg) extract was solubilized in a 10 milliliter (mL) volumetric flask employing absolute methanol as the solvent. Subsequently, a 5 microliter ($\mu$L) aliquot of this solution was introduced into

the UPLC-MS apparatus. The configuration of the analytical settings was set to positive ion mode, and spectra were obtained within a mass range spanning from m/z 120 to 1000. The chromatogram processing and compound identification were carried out using Mass Lynx version 4.1 software developed by Waters, based in Massachusetts, USA. Additionally, the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was utilized for this purpose. The determination of a compound's precision was established by comparing MS/MS fragments, with a tolerance for discrepancies of fewer than 5 parts per million (ppm) (Mutiah et al., 2019a).

#### 2.2.3 Oral Bioavailability Screening

The study employed the Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) database, accessible at https://admetmesh.scbdd.com/, to assess crucial parameters of the chosen compounds. These parameters included Caco-2 cell permeability, human intestinal absorption (HIA), and oral bioavailability (OB) limits F (F-20%, F-30%).The user's text does not provide any information to rewrite in an academic manner (Dong et al., 2018).

#### 2.2.4 Identification of Potential Targets for Colorectal Cancer

Identification of Potential Targets for Colorectal Cancer A crucial element of pharmaceutical research entails the prediction of interactions between drugs and specific targets. The gene targets linked to the active compounds were identified by the utilization of LC-MS/MS analysis in the LPBE. The Gene Cards database (https://www.genecards.org/) was employed for this purpose. Concurrently, the DisGeNET database (https://www.disgenet.org) was utilized to investigate gene targets associated with colon cancer. Following that, a network pharmacology study was performed using Cytoscape software version 3.9.1 in order to acquire a comprehensive understanding of the interactions between active drugs and gene targets, as well as the outcomes pertaining to disease-gene targets (Liu et al., 2018).

#### 2.2.5 The Construction of Pharmacological Networks and Protein-Protein Interactions

The process of establishing pharmacological network linkages, which involve active substances, target genes, and illnesses, was executed with Cytoscape version 3.10. To conduct a more in-depth examination, we identified and analyzed gene targets that were common to both active drugs and illnesses. This study was performed using the STRING platform version 12.0, which can be accessed at https://string-db.org/. The establishment of the Protein-Protein Interaction (PPI) network involved the inclusion of shared target proteins, with a minimum interaction score threshold of 0.400. The objective of doing PPI network analysis was to explore biological phenomena by scrutinizing functional annotations associated with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Yang et al., 2023).

#### 2.2.6 The Analysis of Gene Ontology (GO) and the Enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways

The targets that were discovered underwent an investigation utilizing Gene Ontology (GO) and functional pathway enrichment. This analysis was conducted using the R programming language. The screening parameters for evaluating functional enrichment data were established at a significance level of $p = 0.05$ and a false discovery rate of $q = 0.05$. The GO analysis was conducted to identify the most noteworthy discoveries pertaining to cellular component (CC), molecular function (MF), and biological process (BP). These findings were then visually represented using R programming language to generate a bubble chart. Furthermore, the bubble diagrams for visual representation were generated using SRPlot (http://www.bioinformatics.com.cn/srplot), using the top thirty KEGG pathway enrichments (Jiang et al., 2020).

#### 2.2.7 Molecular Docking

AutoDock Vina is commonly employed in molecular docking to achieve optimal positioning and binding interactions between ligands and proteins. The present study involved the docking procedure of the oxoberberine molecule with six specific target receptors, namely BAX (PDB ID: 2YXJ), BCL2 (PDB ID: 2W3L), and STAT3 (PDB ID: 6NJS). The ligand structures were acquired from the PubChem database, whilst the receptor structures were sourced from the Protein Data Bank. To conduct the initial validation, the receptors were re-docked with their respective original ligands, taking into account an RMSD parameter of less than 2.0 Å. The outcomes of the compound docking were evaluated and ordered according to the affinity energy values. The visualization of the ligand-receptor interactions was conducted using Biovia Discovery Studio and PyMol 19 software (Mutiah et al., 2019b).

## 3. RESULTS AND DISCUSSION

### 3.1 Metabolite Profiling

17 compounds have been found in the 70% ethanol extract from the stem of Lansium parasiticum (LPBE) through the use of the UPLC-QtoF-MS/MS device (Table 1). The main components in the LPBE are moronate acid with a concentration of 14.29%, 4-Morpholineacetic acid with 12.2%, and Ursolic aldehyde with approximately 8.37%. Chromatogram of Lansium parasiticum bark extract using UPLC-QToFMS/MS method has been presented in Figure 1.

Each peak in the chromatogram in Figure 1 indicates the presence of a single compound. The analysis results of the chromatogram indicate the identification of 17 compounds, as shown in Table 1. These compounds are categorized into several groups, including 7 alkaloid compounds detected at peaks 1, 2, 3, 7, 8, 11, and 12. Additionally, there are 2 coumarin compounds at peaks 4 and 5, as well as 1 terpenoid compound, dukunolide E, at peak 6. There are also 2 triterpenoid compounds, dukunolide D at peak 9 and moranic acid

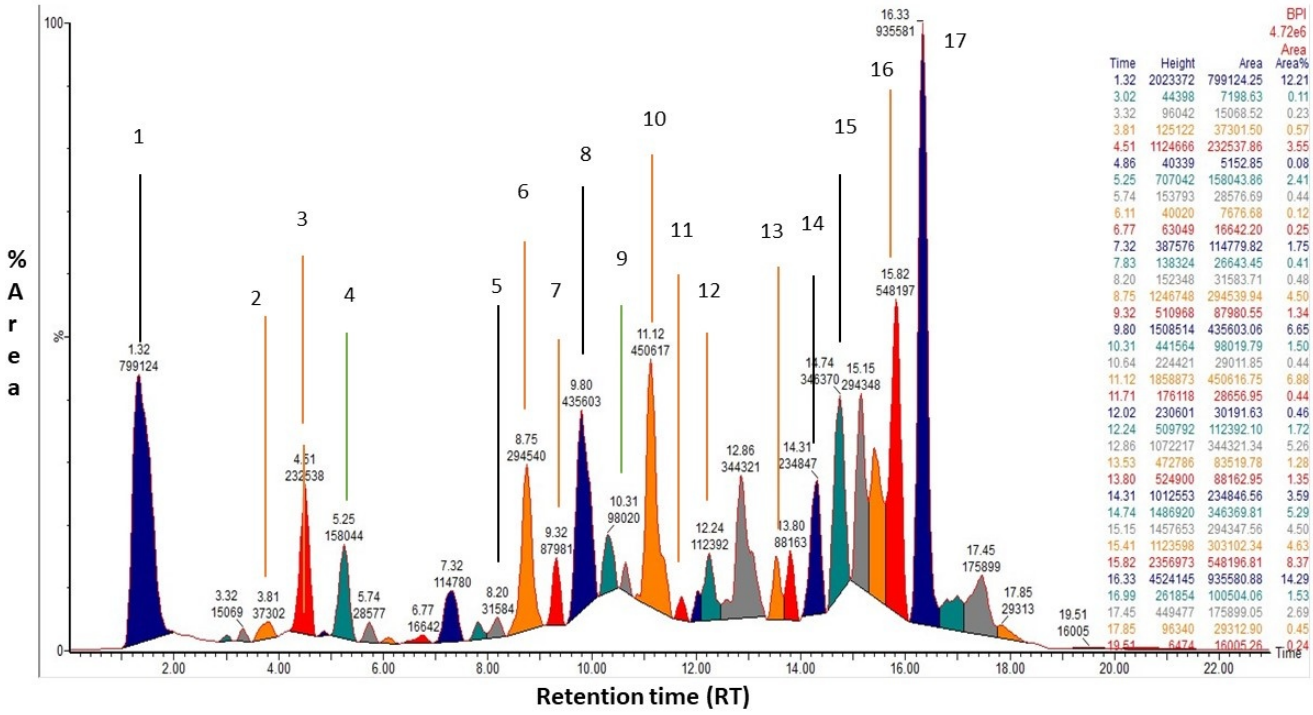


**Figure 1.** Chromatogram of *Lansium parasiticum* Bark Extract using UPLC-QToFMS/MS method. It was C18 Stationary Phase; the Mobile Phase was Water/Formic Acid [99.9/0.1 (v/v)] and Acetonitrile/Formic Acid 99.9/0.1 (v/v). Each Chromatogram Peak Indicated One Compound

at peak 17. Furthermore, 1 flavonoid compound, namely 3,6-Dimethylmangostin, was identified, and finally, there are 4 steroid compounds indicated by peaks 13 through 16.

Subsequently, oral bioavilability screening was performed using Caco-2 cell permeability, human intestinal absorption (HIA), and Oral Bioavilibility (OB) limits F (F-20%, F-30%). Screening results showed that 17 compounds meet the parameter of oral bioavailability (Table 2).

### 3.1.1 Gene Target Potensial and Protein-Protein Interaction

In an attempt to find potential target gene compounds in LPBE for colon cancer treatment using GeneCards, it has been revealed that of the 17 compound contained in the Lansium parasiticum bark extract, they are connected to 301 potential target genes. On the other hand, the target genes associated with colon cancer included 272 genes, including Stage II Colon Cancer (CUI: C0278479), Stage I Colon cancer AJCC v7 (CUI: C3146257), Stage III Colon Carcinoma AJCCv8 (CUI: C4525119), Stade III Colonic Cancer CUI:C0278480, Stage IV Colon Cancers AJCCV7 (SUI: c3145254), Stage V III Colonial Cancer AJCC V8 (SII: c4525124) based on data from Disgenet. From a Venn diagram analysis comparing the target gene of a phytochemical compound with the target disease gene, it was found that there were 13 potentially overlapping genes (Figure 1, Table 3). Further, of these 13 target genes, further analysis related to the pharmacological tissue was carried out using the Cytoscape device (Figure 2).

The results of the pharmacological network analysis of the compounds in LPBE with the colon cancer target gene showed that of the 17 existing compound, there were 3 compounts that targeted 6 genes with a score of >7. Three of them were Ganoderic acid SZ with a target gene, Ursolic aldehyde with 3 target genes, and Moronic acid 1 target gene.

### 3.1.2 Gene Ontology Analysis and Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment

The application of genetic ontological analysis reveals that the substances present in LPBE exert an influence on many biological processes, molecular functions, and cellular components. The biological bubble diagram illustrates the top ten biological processes that are impacted by the substances in the LPBE, as depicted in Figure 3a. The biological process refers to the series of events and activities that occur within living organisms, involving various physiological and biochemical mechanisms. The highest degree of significance is attributed to the regulation of mitochondrial membrane permeability, which entails the intricate interplay among three specific genes. In Figure 3b, the cellular component analysis reveals the ten most prominent cell components influenced by the substance in question. The

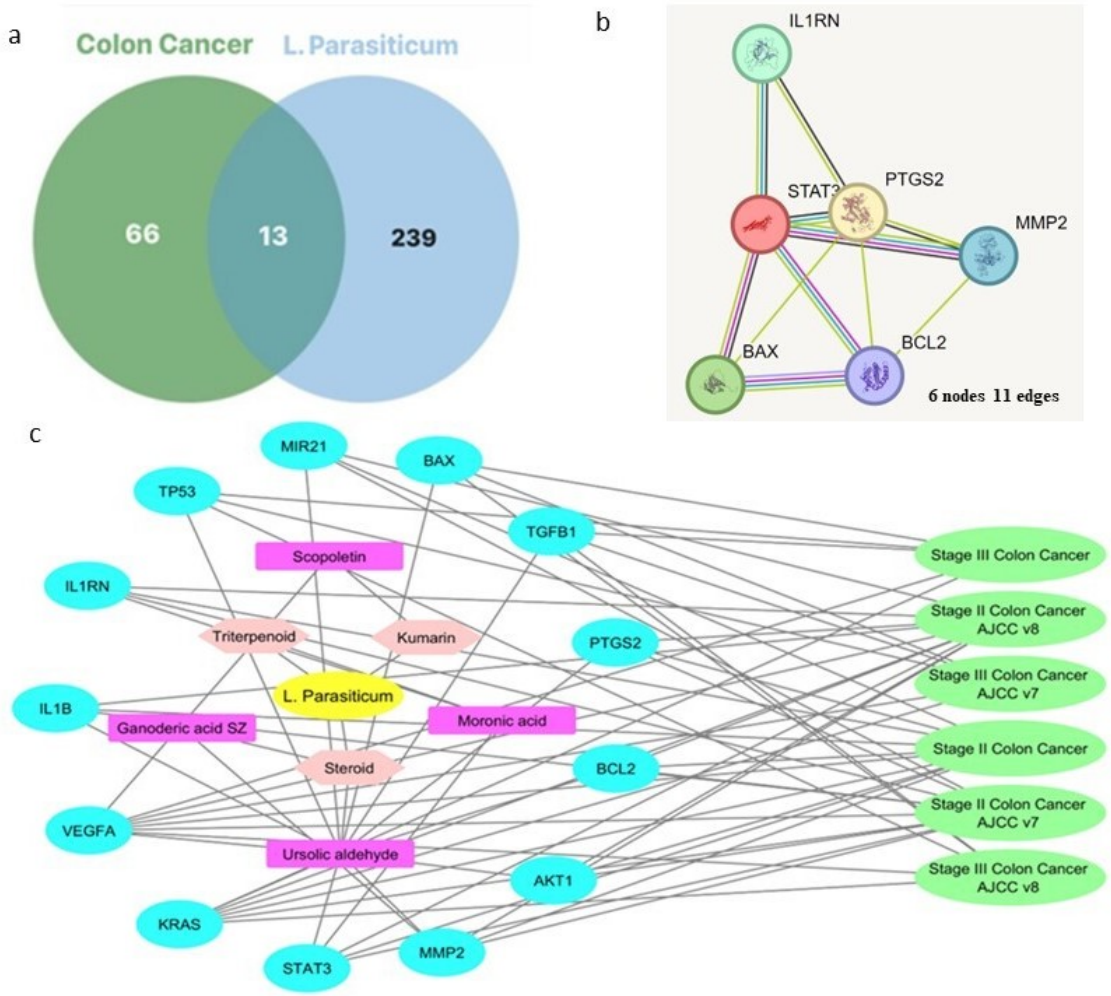


**Figure 2.** a) Venn Diagram of Gene Targets for Compounds in *Lansium parasiticum* Bark Extract and Colon Cancer Disease Target Genes, Including Four Types: Stage II Colon Cancer, Stage II Colon Cancer AJCC v7, Stage II Colon Cancer AJCCv8, Stage III Coloncancer, Stage II Colon Cancers AJCCCv7, Stage III Colon Cancers AjCCv8. b) Protein-Protein Interaction (PPI) of Compounds in *Lansium parasiticum* Bark Extract Involved in the Biological Pathway of Colon Cancer Treatment. c) Network Topology of Compounds in *Lansium parasiticum* Bark Extract with Target Genes (Number of Nodes: 27, Number of Edges: 68). Yellow: Plant Name, Pink: Compound Group, Oak: Bioactive Component, Young Green: Disease, Young Blue: Target Protein

cellular component with the greatest level of elevation is the outer membrane of the organelle, namely the outer diaphragm, together with the nuclear envelope that encompasses three distinct target genes.

Biological process Regulation of mitochondrial membrane permeability has the highest value and involves interaction of 3 genes on cellular components (Figure 3d) showing the 10 most potential cell components affected by this compound. The best cellular component is the organella outer membarne, the external membarne and the nuclear envelop with each involving 3 target genes. On the molecular function (Figure 3c) there are 2 moleculares functions with the most significant influence ($p<0.001$) namely the death domain binding and the BH domain bending with each involved 2 target gene.

The KEGG enrichment analysis (Figure 4a and 4b) found that there are two potential pathways in the treatment of colon cancer affected by the three compounds in LPBE: Ganoderic acid SZ, Ursolic aldehyde, and Moronic acid. The two pathways are EGFR tyrosine kinase inhibitor resistance (hsa01521) and apoptosis (hse04210). The EGFR Tyrosine Kinase Resistance pathway in Figure 5 shows that there are three important potential target genes on this pathway: BCL2, BAX, STAT3. Three of these genes capture the potential target gene of the compound 2 in the LPBE.

When EGFR is activated, it triggers a signal pathway that leads to cell growth, division, and survival. In many cases of cancer, including colon cancer, EGFR's overactive, causing uncontrolled growth of cancer cells (de Castro-Carpeño et al., 2008). The EGFR tyrosine kinase inhibitor works by inhibiting the activity of these enzymes, thereby stopping the signals that

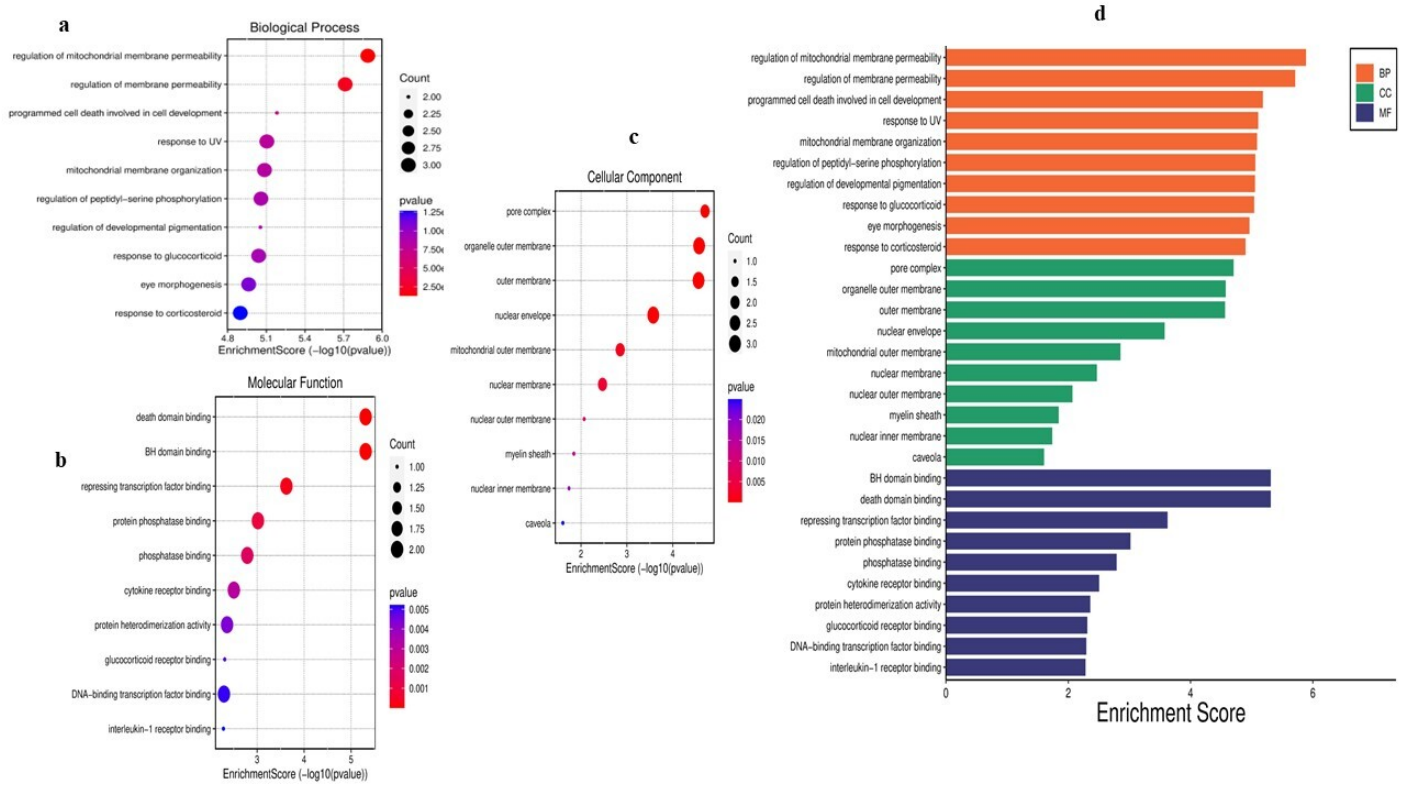


**Figure 3.** Analysis of Genetic Enrichment Ontology and KEGG Pathways; a) Bubble Enrichment Diagram of the 10 Biological Processes with the Highest Potential; b) Bubble Diagram for the 10 Molecular Functions with the Most Potential; c) Bubbles Diagram on the 10 Cellular Components with the Greatest Potential. d) An Enrichment GO Bar Diagram for Biological Processes, Cellular Components, and Molecular Functions

**Table 1.** The Results of Metabolite Identification of *Lansium parasiticum* Bark Extract using UPLC Qtof MS/MS Method

| NO | Rt | %Area | Measured Mass | Calculated Mass | Formula | Name | Groups |
|---|---|---|---|---|---|---|---|
| 1 | 1.324 | 12.2% | 146.0821 | 146.0817 | $C_6H_{12}NO_3$ | 4-Morpholineacetic Acid | Alkaloid |
| 2 | 3.826 | 0.57% | 310.093 | 310.0927 | $C_{14}H_{16}NO_7$ | 7-Deoxypancratistatin | Alkaloid |
| 3 | 4.529 | 3.5% | 352.1613 | 352.1608 | $C_{14}H_{26}NO_9$ | Validoxylamine B | Alkaloid |
| 4 | 5.253 | 2.41% | 193.0498 | 193.0501 | $C_{10}H_9O_4$ | Scopolet's | Coumarin |
| 5 | 8.199 | 0.48% | 175.1489 | 175.1487 | $C_{13}H_{19}$ | 1,1,6- Trimethyltetral | Coumarin |
| 6 | 8.747 | 4.50% | 485.1828 | 485.1812 | $C_{26}H_{29}O_9$ | Dukunolide E | Terpenoids |
| 7 | 9.296 | 1.34% | 486.213 | 486.2128 | $C_{26}H_{32}NO_8$ | Saccharothriolide D | Alkaloid |
| 8 | 9.802 | 6.65% | 517.2096 | 517.2074 | $C_{27}H_{33}O_{10}$ | Ananolignan F | Alkaloid |
| 9 | 10.639 | 0.44% | 469.1882 | 469.1862 | $C_{26}H_{29}O_8$ | Dukunolide D | Triterpenoids |
| 10 | 11.124 | 6.88% | 439.2137 | 439.2121 | $C_{26}H_{31}O_6$ | 3,6-Dimethylmangostin | Flavonoid |
| 11 | 12.024 | 0.46% | 618.2916 | 618.2888 | $C_{28}H_{40}N_7O_9$ | Rotigaptide | Alkaloid |
| 12 | 12.242 | 1.72% | 627.2805 | 627.2779 | $C_{30}H_{39}N_6O_9$ | Tripeptide-based inhibitor | Alkaloid |
| 13 | 13.535 | 1.28% | 453.3378 | 453.3402 | $C_{30}H_{45}O_3$ | Ganoderic acid SZ | Steroid |
| 14 | 14.309 | 3.59% | 335.2578 | 335.2586 | $C_{21}H_{35}O_3$ | Tetrahydrodeoxycorticosterone | Steroid |
| 15 | 14.744 | 5.29% | 471.3492 | 471.3474 | $C_{30}H_{47}O_4$ | Enoxolone | Steroid |
| 16 | 15.82 | 8.37% | 441.3753 | 441.3733 | $C_{30}H_{49}O_2$ | Ursolic aldehyde | Steroid |
| 17 | 16.326 | 14.29% | 455.3551 | 455.3525 | $C_{30}H_{47}O_3$ | Moronic acid | Triterpenoids |

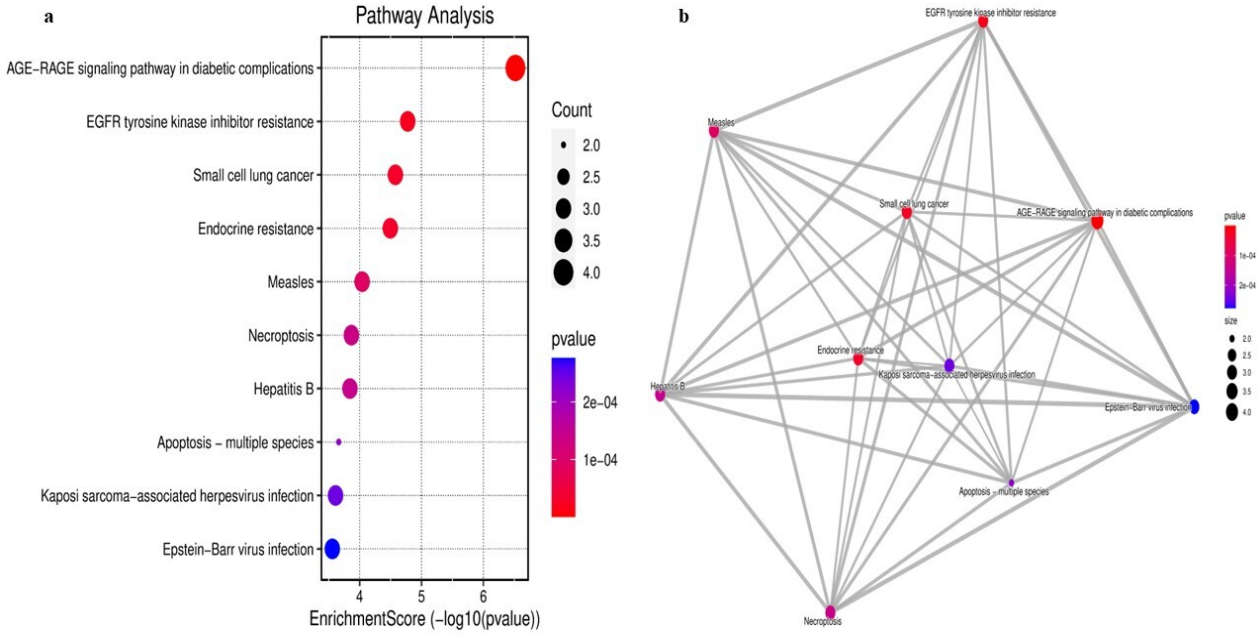


**Figure 4.** Genetic Enrichment Ontology Analysis and KEGG Path; a) Bubble Diagram Analysis of KegG 10 Pathway Potential Compounds in Colon Cancer Inhibition b). Inter-pathway Interaction Diagram Affected by Compounds on *Lansium parasiticum* Bark Extract

**Table 2.** Absorption and Bioavailability Profile of LPBE Compounds

| Compounds | Caco-2 | HIA | F20 | F30 |
|---|---|---|---|---|
| 4-Morpholineacetic Acid | -5.726 | <30% | <20% | <30% |
| 7-Deoxypancratistatin | -5.424 | <30% | <20% | <30% |
| Validoxylamine B | -5.505 | <30% | <20% | <30% |
| Scopolet's | -4.725 | <30% | <20% | <30% |
| 1,1,6-Trimethyltetralin | -4.595 | <30% | <20% | <30% |
| Dukunolide E | -5.323 | <30% | <20% | <30% |
| Saccharothriolid e D | -5.799 | <30% | <20% | <30% |
| Ananolignan F | -4.806 | <30% | <20% | <30% |
| Dukunolide D | -5.341 | <30% | <20% | <30% |
| 3,6-Dimethylmangostin | -4.823 | <30% | <20% | <30% |
| Rotigaptide | -6.889 | <30% | <20% | <30% |
| Tripeptide-based inhibitor | -6.086 | <30% | <20% | <30% |
| Ganoderic acid SZ | -5.039 | <30% | <20% | <30% |
| Tetrahydrodeoxycorticosterone | -4.765 | <30% | <20% | <30% |
| Enoxolone | -5.415 | <30% | <20% | <30% |
| Ursolic aldehyde | -4.990 | <30% | <20% | <30% |
| Moronic acid | -5.272 | <30% | <20% | <30% |

cause the growth of cancer cells (Rego et al., 2010).

The use of EGFR tyrosine kinase inhibitors (TKIs) in the treatment of colon cancer has shown significant results, especially in patients with certain genetic mutations that make cancer cells more susceptible to this treatment. TKIs such as cetuximab and panitumumab have been used to treat metastatic colon cancer, in patients who have certain types of mutations in the KRAS, NRAS, and BRAF genes, which do not show resistance to this therapy (Laurent-Puig et al., 2009; Siddiqui and Piperdi, 2010).

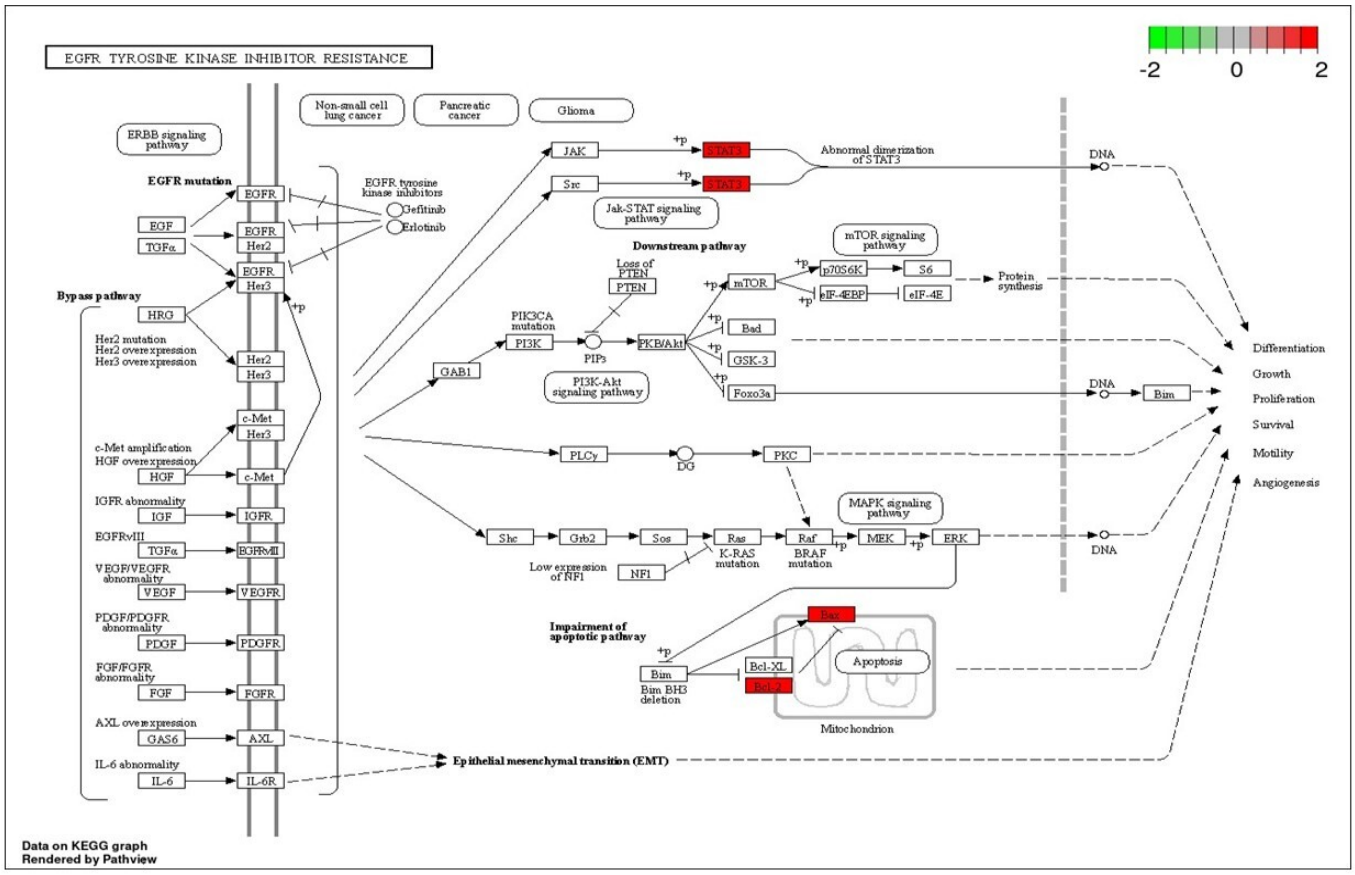


**Figure 5.** EGFR Tyrosine Kinase Inhibitor Resistance (hsa01521) Involving 3 Potential Target Genes (Red Mark) in the Treatment of Colon Cancer with Extract of the *Lansium parasiticum* Bark

**Table 3.** The Target Gene of the Compound in *Lansium domesticum* and Relevance Score of Target Gene (Relevance score>7)

| Compound | Gen Symbol | Description | Gifts | Relevance score |
|---|---|---|---|---|
| Ganoderic acid SZ | MMP2 | Matrix Metallopeptidase 2 | 57 | 14.66635132 |
| Ursolic aldehyde | BCL2 | BCL2 Apoptosis Regulator | 55 | 9.889139175 |
| | BAX | BCL2 Associated X, Apoptosis Regulator | 55 | 7.488071918 |
| | PTGS2 | Prostaglandin-Endoperoxide Synthase 2 | 53 | 7.15674305 |
| Moronic acid | STAT3 | Signal Transducer And Activator Of Transcription 3 | 58 | 13.66884232 |
| | IL1RN | Interleukin 1 Receptor Antagonist | 54 | 11.44108963 |

### 3.1.3 Docking Molecular

Molecular docking results revealed that Ursolic aldehyde, and Moronic acid showed high affinity to BCL2, BAX, and STAT3 receptors. Figure 6a and 6b shows that the two compounds have a higher affinity to their ligaments, indicated by lower affinities than their ligates. The higher the affinity, the more stable the bond between the drug and the receptor (Patil et al., 2020). In the context of interaction with receptors on this molecular docking, the compound showed best activity on pathways related to apoptosis and EGFR tyrosine kinase inhibitor resistance.

Pharmacological network analysis showed that it works on the EGFR tyrosine kinase resistance and apoptosis pathway by targeting the BCL2, BAX, STAT3 genes. Bcl2 is a gene that plays a role in regulating the balance between cell survival and apoptosis. The results of the analysis were supported by insilique validation with molecular docking between the ursolic aldehyde compound and the BCL2 protein (PDB ID;2W3L) which showed that the compounds had excellent affinities with higher energy generated than the active ligans. (PDB ID: 2W3L).

In addition to affecting the target gene BCL2 on the EGFR path, the ursolic acid compound is known to affect the Bax gene. Bax, on the other hand, is a pro-apoptotic gene (Radha

 

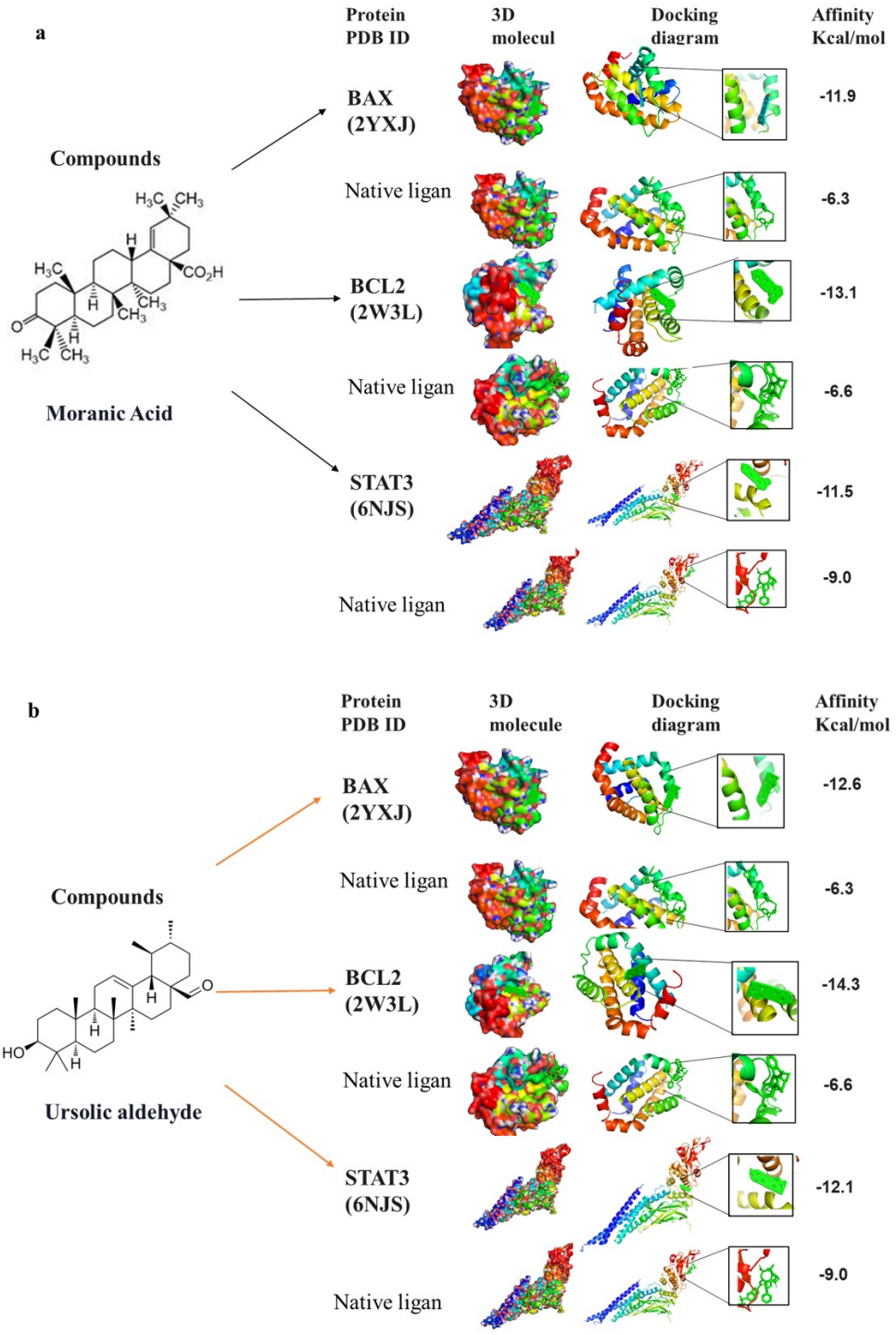


**Figure 6.** Docking Molecular Compounds Moronic Acid (a) and Ursolic Aldehyde (b) with Target Proteins BAX (PDB ID:2YXJ), BCL2 (P DB ID:2W3L) and STAT3 (PDB ID:6NJS)

and Raghavan, 2017; Khodapasand et al., 2015). The protein produced by this gene plays a role in inducing apoptosis (Campbell and Tait, 2018). Ursolic acid can increase Bax's expression or activity, thus promoting cancer cell death. Insilico validation supports the results of a pharmacological network where the compound has a higher affinity to the BAX receptor (PDB ID:2YXJ) compared to its native ligation.

Pharmacological network analysis suggests that moronic acid compounds can influence the expression or activity of the STAT3 gene through the EGFR pathway. STAT 3 inhibition

by moronic acids can be an important mechanism in the anti-cancer effects of these compound (Ma et al., 2020). By reducing STAT3-activity, moronic acid can inhibit the growth of cancer cells and promote apoptosis, as well as reduce inflammation and angiogenesis that often support tumor growth. Validation by insilico method confirms findings from pharmacological network analysis, suggesting that this compound has a stronger tendency to bind to the STAT3 receptor than its original ligan.

Overall, the discussion of this research reveals that Lansium parasiticum bark extract (LPBE) contains 17 active compounds, predominantly belonging to the alkaloid group, with moronic acid, 4-Morpholineacetic Acid, and ursolic aldehyde as the main components. Pharmacological analysis indicates that these compounds have effects on the EGFR tyrosine kinase and apoptosis pathways, targeting key genes such as BCL2, BAX, and STAT3. Furthermore, ursolic aldehyde shows potential in enhancing apoptosis in colorectal cancer cells by reducing BCL2 expression and increasing BAX activity. These findings are supported by in silico validation, demonstrating high affinity of ursolic aldehyde towards BCL2 and BAX receptors. On the other hand, moronic acid is found to affect the STAT3 gene, potentially triggering anti-cancer mechanisms by inhibiting cancer cell growth and stimulating apoptosis. Thus, this research provides insights into the therapeutic potential of LPBE in the treatment of colorectal cancer.

However, the study has limitations as it is primarily conducted through bioinformatics and in silico validation. Further validation, both in vitro and in vivo using animal models, is still necessary. Additionally, for its development into a phytopharmaceutical product, clinical trials involving human subjects are required.

## 4. CONCLUSIONS

LPBE contains 17 active compounds, dominant in the alkaloid group, with moronic acid, 4-Morpholineacetic Acid, and ursolic aldehyde as the main components. Pharmacological analysis showed the effects of these compounds on the EGFR tyrosine kinase and apoptosis pathways, targeting genes such as BCL2, BAX, and STAT3. Specifically, ursolic aldehyde has the potential to enhance apoptosis in colon cancer cells through a decrease in BCL2 expression and increased BAX activity, confirmed by insilico validation that showed high affinity with BCL2- and BAX-receptors. Moronic acid, on the other hand, affects the STAT3 gene, promoting anti-cancer mechanisms by inhibiting cancer cell growth and triggering apoptysis. The findings highlight the therapeutic potential of LPBE in the treatment of cancer.

## 5. ACKNOWLEDGMENT

The researchers expressed their gratitude to Maulana Malik Ibrahim Malang State Islamic University Research and Public Service Institute who has funded this investigation with grand number 0910/FKIK/05/2023, so that it can run well and get results that can benefit the academic world.

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