Network pharmacology of black cumin (*Nigella sativa* L.) as a candidate of OMAI in colorectal cancer: in silico study

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**ABSTRACT** Colorectal cancer is the third most common cancer globally and the second leading cause of cancer-related deaths. The management of colorectal cancer requires consideration of various factors due to the non-selectivity of drugs, meaning that highly effective treatment with lower side effects is needed. Black cumin (*Nigella sativa* L.) contains thymoquinone and various other metabolites with potential as anticancer effects. The involvement of various genes and the difficulty of drug development have led to a shift in the drug development paradigm towards plant-based medicine that is both multicomponent and synergistic in supporting the resulting pharmacological effects. Network pharmacology can predict the synergistic effect of a multicomponent approach. This study aimed to predict the network pharmacology of black cumin as a candidate for OMAI (“Obat Modern Asli Indonesia”, Indonesian-origin modern medicine) in colorectal cancer. This research was an in silico study using various ethnobotanical databases and software. The results show that seven metabolites in black cumin are correlated with ten surface receptor proteins, 30 intracellular proteins, and mechanisms involving six colorectal cancer signaling pathways. This result indicates that *Nigella sativa* L. has potential in OMAI and can be a reference for the development of cancer treatment, especially for colorectal cancer.

**KEYWORDS** colorectal cancer; in silico; natural remedies; network pharmacology; *Nigella sativa*

1. Introduction

Cancer is a malignant, autonomous, and uncontrolled cell proliferation process that can spread (metastasize) to surrounding organs. The number of cases and deaths from cancer is predicted to grow rapidly, and the increasing population, age, and lifestyle changes can increase cancer risk. This problem affects the quality of human resources due to the impact of cancer on various aspects of life (Singer 2018). Colorectal cancer is the third most common cancer globally and is the second leading cause of cancer-related deaths (Florescu-Țenea et al. 2019). Colorectal cancer can occur sporadically (70%), familial clusters (20%), and hereditary syndromes (10%) such as familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPPC) and are associated with a poor environment and lifestyle (Recio-Boiles and Cagir 2021). According to the Ministry of Health of the Republic of Indonesia (2018), the management of colorectal cancer is multidisciplinary and requires various factors. Local procedures have a higher recurrence rate, surgical therapy is less effective, especially for metastatic cancer, and chemotherapy and monoclonal antibodies have different side effects. This problem is mainly due to the non-selectiveness of the drugs used because they also damage healthy cells, so drugs with high effectiveness and low side effects are needed.

Indonesia consists of thousand islands with abundant biodiversity, so it has enormous potential as a source of new drug discovery (Sutiono et al. 2017). OMAI (“Obat Modern Asli Indonesia”, Indonesian-origin modern medicine) are natural and authentic Indonesian medicines that already have scientific evidence of their safety and efficacy, consisting of Standardized Herbal Medicines and Phytopharmaceuticals (National Agency of Drug and Food Control of the Republic of Indonesia 2020). One of the natural ingredients in Indonesia that have the potential as an anticancer is black cumin (*Nigella sativa* L.). Thymoquinone (TQ), as the main phytochemical in *Nigella sativa* L., can inhibit the growth of colorectal cancer cells, increase cell morphology changes and induce apoptotic (Kooti et al. 2016; Hosseinzadeh et al. 2017).

Most diseases involve different groups of genes. On the other hand, drug development costs a lot and takes a long time (Gunev et al. 2016). This problem has caused the drug development paradigm to change its focus from conventional drugs that are one drug-one target to plant-based medicine that is both multicomponent and synergistic in supporting the resulting pharmacological effects. Network pharmacology can predict the synergistic effect of a multicomponent approach. This study aimed to predict the network pharmacology of black cumin as a candidate for OMAI (“Obat Modern Asli Indonesia”, Indonesian-origin modern medicine) in colorectal cancer. This research was an in silico study using various ethnobotanical databases and software. The results show that seven metabolites in black cumin are correlated with ten surface receptor proteins, 30 intracellular proteins, and mechanisms involving six colorectal cancer signaling pathways. This result indicates that *Nigella sativa* L. has potential in OMAI and can be a reference for the development of cancer treatment, especially for colorectal cancer.

**KEYWORDS** colorectal cancer; in silico; natural remedies; network pharmacology; *Nigella sativa*
based treatments containing various chemical compounds with various targets (multicomponent – network targets) (Syahrir et al. 2016). The combination of active compounds in multicomponent drugs can be synergistic to support the resulting pharmacological effects. The synergistic effect on the multicomponent approach can be carried out quickly and gives more promising results through various in silico computational methods. One of them is network pharmacology, which explains the principles of network theory between compounds and biological systems and from the results in in vitro and in vivo testing for drug development (Yi et al. 2018).

A preliminary study is very important to provide an initial view in reaching the right conclusions and efficient preclinical research. We use several online databases to predict the compound content and the mechanism of action described based on the protein-structure relationship of the compound through computer prediction. This study aims to predict the network pharmacology of black cumin (Nigella sativa L.) as a candidate for OMAI in colorectal cancer. Our research can also serve as a reference for the development of cancer drugs based on target genes to support the creation of new medicines or innovations in cancer treatments that are safe and effective.

2. Materials and Methods

2.1. Secondary metabolites of black cumin (Nigella sativa L.)

The metabolites used in this study were obtained from the KNApSAcK Family database (http://www.KNApSAcKFamily.com/) and Dr. Duke’s Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search). The chemical structure of black cumin metabolites was validated by using PubChem database (https://pubchem.ncbi.nlm.nih.gov) and were generated by Marvin Sketch software.

2.2. Prediction of biological activity and protein target of black cumin (Nigella sativa L.) metabolites

The Way2Drug PASS Online (http://way2drug.com/passonline/) and Swiss Target Prediction (http://www.swistargetprediction.ch/) were used to predict the biological activity and target proteins of black cumin metabolites in colorectal cancer. All accessible targets were restricted to Homo sapiens.

2.3. Pathway analysis in colorectal cancer

The protein-protein interactions involved in the colorectal cancer pathway were analyzed using the STRING database (https://string-db.org/), GeneCards (https://www.genecards.org), and PubChem (https://pubchem.ncbi.nlm.nih.gov).
and KEGG Pathway (https://www.genome.jp/kegg/pathway.html). Analysis of metabolites-protein interactions involved in the colorectal cancer pathway was carried out using STITCH database (http://stitch.embl.de/).

2.4. Metabolites-target-pathway network construction

Visualization of network pharmacology the black cumin (N. sativa L.) was carried out using Cytoscape v.3.8.2 software.

2.5. Methods

Prediction of the metabolites contained in black cumin was carried out through the KNApSAcK Family and Dr. Duke’s Phytochemical and Ethnobotanical, then generated by Marvin Sketch software. The metabolites’ biological activity and target proteins were predicted through Way2Drug PASS Online and Swiss Target Prediction. GeneCards and KEGG Pathway were used to determine the involvement of target proteins with colorectal cancer. The metabolites mechanism of action was predicted through STRING, thus referring to biological activity in the KEGG Pathway. The interaction between the metabolites and their target proteins was predicted through STITCH and visualized through the Cytoscape software to describe a network pharmacology. The research flow chart can be seen in Figure 1.

3. Results and Discussion

Prediction of metabolites contained in black cumin through KNApSAcK Family and Dr. Duke’s Phytochemical and Ethnobotanical yielded ten metabolites in the seed portion and five metabolites obtained in the Nigella sativa L. seed oil portion (Table 1). According to Corso et al. (2020), KNApSAcK is a comprehensive database containing information on the relationship between metabolites, biological activities, and species. Dr. Duke’s Phytochemical and Ethnobotanical database has the advantages of free access at no cost and the existence of supporting references that correlate with ethnomedicinal data which shows high significance (Savithramma et al. 2016). Black cumin seeds have an important history in traditional medicine practices, especially in South and Southeast Asia, Arabia, Africa and the Mediterranean, treating several diseases including cancer (Khan et al. 2017).

Prediction of the structure of the selected metabolites through Marvin Sketch is shown in Figure 2. The structure of metabolites varied from simple organic compounds, long-chain lipids, to glucosides.

The biological potential of the metabolites was predicted through the Way2Drug PASS Online database accompanied by the value of the activity probability (Pa) or the likelihood of “becoming active” (Juan et al. 2020). Way2Drug PASS Online provides predictions of biological activity of more than 4,000 types of biological activity with an average of 95% accuracy based on the structure of organic compounds, both existing and new, enabling screening of unpromising compounds at the earliest stage of a study (Filimonov et al. 2014). All metabolites contained in black cumin, except nigellidine, have biological potential associated with cancer through various activities with Pa>0.7, which means that the probability of finding activity in the experiment is quite high and the predicted metabolites are likely to be close to the structure

| Compound Name | CAS_ID | SMILES | Plant Part |
|---------------|--------|---------|------------|
| m-Thymol      | 89-83-8| c1cc(cc1)CC(=O)CIC   | Seed Oil   |
| Oleic acid    | 112-80-1| CCCCCCCCC/C=C/C=CCCCCCCC(=O)O   | Seed       |
| Myristicin    | 607-91-0| c12c(ccc1)CC(=O)OCO2  | Seed Oil   |
| p-Cymene      | 99-87-6 | c1cc(ccc1)CC(=O)OC   | Seed       |
| beta-Amyrin   | 559-70-6| C1C=C(C(=O)C=C(C1=O)C)C(C)C   | Seed       |
| Linolenic acid| 463-40-1| OC(CCCCCCC/C=C/C=CCCCCCCC(=O)O)O   | Seed       |
| Thymoquinone  | 490-91-5| C1=C(C(=O)C=C1)O=CICIC   | Seed Oil   |
| Kaempferol 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside | 197250-98-9 | c1cc(c(c1)O)cc(c(c1)O)cc(c(c2)O)O | Seed Oil |
| Quercetin 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside | 197250-97-8 | c1cc(c(c1)O)cc(c(c1)O)cc(c(c2)O)O | Seed Oil |
| Quercetin 3(6)"-Feruloylglucosyl-(1→2)-galactosyl-(1→2)-glucoside | 197294-29-4 | c1cc(c(c1)O)cc(c(c1)O)cc(c(c2)O)O | Seed Oil |
| Nigellidine   | 98063-20-8| c12d[n+](3h1c1c1O)cCCCCCC3ccc2O/C | Seed       |
| Nigellidine   | 120993-86-4| c12d[3n1c1cc(c1)O]CCCCCCccc2c2O/C | Seed       |
| Nigelline     | 4594-02-9 | c1cc(=c2ccc1)(c2)OCOCOC   | Seed       |
| Carvone       | 99-49-0  | C1=C(C(=O)C=C1)c1c1O   | Seed       |
| Nigellidine 4-O-sulfite | 103262-86-8 | c12d[3n1c1cc(c1)O]CCCCCCccc2O51O(=O)O/C | Seed       |
FIGURE 2 Prediction of the structure of metabolites in *Nigella sativa* L. with Marvin Sketch.

analogs drugs of known (Figure 3). The highest prediction was found in the quercetin 3-(6‴-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside with a probability of 0.997 on free radical scavenger activity and 0.996 on chemopreventive and antimutagenic activity. Their other activities include HIF1A expression inhibitor, JAK2 expression inhibitor, TP53 expression enhancer, apoptotic agonist, transcription factor stimulant, antineoplastic, anti-inflammatory, anticarcinogenic, etc.

The results of target protein prediction through the Swiss Target Prediction database showed that the metabolite contents in black cumin have many possibilities of targeting various proteins from various classes in the body, including those involved in cancer. The proteins analyzed through the GeneCards database resulted a probability>0 (Table 2). This value is derived from the target score range of 0–1 which describes the similarity of the prediction to be true. The greater the probability, the greater the chance of prediction accuracy (Gfeller et al. 2014). The user-friendly graphical interface makes Swiss Target Prediction often used in similar studies (Daina et al. 2019). According to Kononenko et al. (2014), GeneCards also features a key interface that enables a better user experience, including improved data and product information consolidation, retaining legacy content and functionality, and easy navigation to other sites. Prediction of target proteins involved in cancer in these metabolites amounted to 192 target proteins, mostly from the kinase class and several other classes such as oxidoreductases, nuclear receptors, hydrolases, etc.

Prediction of colorectal cancer signaling pathways was obtained from KEGG Pathway. The construction of the interaction network between the target proteins was carried out through STRING and focused on proteins with a minimum interaction score>0.400 (medium confidence) from the range 0–1. This value indicates a biologically significant relationship (interaction) compared to the accompanying evidence. The higher the interaction score, the more biologically meaningful the interaction is. KEGG is a metabolic pathway database containing 16,568 compounds and provides a reference pathway for predicting metabolic pathways in an organism (Altman et al. 2013). The high coverage, ease of use, and consistent scoring system, as well as additional access features via APIs and applications make STRING widely used in bioinformatics studies to analyze protein-protein interactions (Szklarczyk et al. 2019). The results of the intersection analysis
showed that the ten target proteins produced are surface receptor proteins (PIK3CA, TGFBR1, EGFR, MAPK1, MAPK3, MAPK8, AKT1, GSK3B, CASP3, MAPK10) that were directly involved in six colorectal cancer signaling pathways (Table 3).

The construction of the interaction network between the black cumin metabolites and its target protein through STITCH was viewed with a confidence view (default) where the thickness of the edges depicted different levels of confidence based on the interaction analysis scores showing differences in the strength of the interaction data support (Figure 4). The score indicates a medium confidence level (>0.400) based on the binding affinity or inhibition constant (Kᵢ), EC₅₀, or IC₅₀. The thicker the interaction line, the stronger the bond between the two interactors. STITCH, a database of protein-chemical interactions derived from various sources, namely prediction of genomic content, experiments, co-expression, text mining, and knowledge of pre-existing protein complexes make it widely used in various kinds of related research (Szklarczyk et al. 2016). The number of intracellular proteins that appear suggests the presence of signal transduction, where the response to changes in chemical signals (extracellular) is converted into intracellular signals.

Based on the interaction analysis, the black cumin metabolites that interact directly with surface receptor proteins were thymoquinone, thymol, myristicin, and oleic acid (Table 4). In addition, there were also four other metabolites in black cumin that do not interact directly with surface receptor proteins but were related to the previous four metabolites, namely linolenic acid, p-cymene, nigellicine, and carvone (Figure 3).

The network pharmacology was visualized with Cytoscape as in Figure 5. The black cumin metabolites interacted with ten surface receptor proteins and 30 intracellular proteins, and the mechanism involved six colorectal cancer signaling pathways (Ras signaling pathway, TGFβ signaling pathway, ERK/MAPK signaling pathway, apoptotic, PI3K-AKT signaling pathway, and Wnt signaling pathway). According to Li et al. (2017), Cytoscape is a friendly and open bioinformatics platform, which demonstrates outstanding performance in both virtualization and manipulation of biological networks, including due to the rich functionality of access to other applications or related databases (Doncheva et al. 2019).

Oleic acid (OA) has a strong relationship with CASP3.
### TABLE 2 Prediction of metabolites target proteins in Nigella sativa L. with Swiss Target Prediction and GeneCards Databases.

| PubChem ID | Compound name                                      | Target genes linked to cancer |
|------------|----------------------------------------------------|------------------------------|
| 6989       | m-Thymol                                           | TRPA1, PTGSI, GABRA1, GABRG2, HTR2B, GABRB3, HTR2C, CA2, CHRM2, FLT3, JAK1, JAK2, PRKCA, AURKA, ESRRG, CDK2, CCNA1, CCNA2, ACHE |
| 445639     | Oleic acid                                         | FABP4, PPARG, PPARA, TERT, FABPS, PPARD, FABP1, FABP3, PTPIN1, PTPIN2, PTGSI, FFAARI, PTGES, TOP1, PREP, PTPN6, ALOX5, CDC25B, CDC25A, AKR1B10, POLB, PDE4D, PTGER2, LTBR, RORC, CYP19A1, NR1H3, NR3C1, CNR1, CD1R, PRKCH, PTGSI, SERPINA6, SHBG, G6PD, PTPFR, PTPIN1, PLAG1B, FNTA, FNTB, ER2R, NPC1LI1, SIGMAR1, CYP17A1, SRD5A2, AR, PGR, MAPK3, PTGER1, PTGER4, ADORA3, NR1I3, TOP2A, CYP26A1, ER3, CHRM2, ACHE, CYP2C19, FABP2, NR1H4, NO5, FFAARI, RORA |
| 4276       | Myristicin                                         | CDK5R1, CDK5, CDK2, CCNA1, CCNA2, CDK9, CCNT1, DRYK3A, METAP2, PDE7A, ADORA2B, ADORA3, ADORA2A, DRYK1B, GRM4, JAK1, JAK2, CHRNA3, PDE5A, JAK3, NUDT1, AKT1, MAPK3K14, GRM5, TNK2, NAMPT, ERBB2, CYP19A1, EPL, HTR2A, HTR2C, LMK1, CDK2, CDKC, CDGC, GABRA3, GABRG2, GABRA1, GABRB3, GABRB2, GABRB1, GABRA5, GABRA2, CCNE1, CDK5, CHK1, KAT5B, KDR, HMX01, PTTRC, STS, NO5, NO5, NO5, TERT, CD3B, TGM2, TYPM, CDK1, TPSO, MAPK8, GSK3B, MET, MAPK10 |
| 7463       | p-Cymene                                           | CYP2A6, ACHE, TAAAR1, PPARA, PTGSI, TRPA1 |
| 73145      | beta-Amyrin                                        | AR, CYP19A1, CHRM2, ACHE, CYP2C19, PTPIN1, NR1I3, ESRI, NPC1LI1, SREBF2, CYP17A1, NR1H3, PTPIN6, PTPIN2, ESRI, PPARA, PPARD, FABP5, PPARD, FABP1, PPARA, POLBI, PDE4D, PTPFR, PLAG1B, AKR1B10, CDC25A, CNR1, FNTA, FNTB, CD81, ADORA3, MAPK3, PTPIN1, NR1I2, UGT2B7, ALOX5, NO52 |
| 5280934    | Linolenic acid                                     | PPARG, PPARA, PPARD, FABR1, FABP1, PTGSI, FABP5, FABP1, TERT, FABP3, FABP5, CYP19A1, NR1I3, ESRI, NPC1LI1, SREBF2, CYP17A1, NR1H3, PTPIN6, PTPIN2, ESRI, PPARA, PPARD, FABP5, PPARA, POLBI, PDE4D, PTPFR, PLAG1B, AKR1B10, CDC25A, CNR1, FNTA, FNTB, CD81, ADORA3, MAPK3, PTPIN1, NR1I2, UGT2B7, ALOX5, NO52 |
| 10281      | Thymoquinone                                       | CYP19A1, CHRM2, ACHE, CYP2C19, PTPIN1, NR1I3, ESRI, NPC1LI1, SREBF2, CYP17A1, NR1H3, PTPIN6, PTPIN2, ESRI, PPARA, PPARD, FABP5, PPARD, FABP1, PPARA, POLBI, PDE4D, PTPFR, PLAG1B, AKR1B10, CDC25A, CNR1, FNTA, FNTB, CD81, ADORA3, MAPK3, PTPIN1, NR1I2, UGT2B7, ALOX5, NO52 |
| 5282156    | Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside | CA2, CA12, NOQ2, ACHE, NOX4, PTGSI, AKR1B1, PDE5A, CD81, ADORA1, TNF, IL2, ALOX5 |
| 16745399   | Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside | CA2, CA12, NOQ2, ACHE, NOX4, PTGSI, AKR1B1, PDE5A, CD81, ADORA1, TNF, IL2, ALOX5, TERT |
| 131751014  | Quercetin 3-[6""-feruloylglucosyl]-(1->2)-galactosyl-(1->2)-glucoside | ACHE, NOX4, CA2, CA12, NOQ2, PTGSI, AKR1B1, CD81, PDE5A, TNF, IL2, ADORA1, TERT |
| 11402337   | Nigellicine                                        | HTR2A, ADRA1D, HTR2B, ADRA1A, HTR2C, ADRA1B, TSPO, JAK3, DBF4, CDC7, MPP3, HPGD, DRYK3A, CDK5R1, CDK5, AR, EGFR, KDR, AURKA, AKT1, SIGMARR1, CSF1R, MMP13, MMP2, MIF, ALOX5, ALOX5, ALOX12, CNR1, MPP9, FGFR1, DRYK1B, GABRA1, CCNE2, CDK2, CCNE1, CDK1, CCNB1, CCNB2, PIM1, MAPKAP2K2, GSK3B, CTXK, CDK2, CDK1, GABRB5, ROCK1, ERK1, AKR1B1, JAK2, CA2, AKR1B10, RBP4, PIK3CG, PIK3CA, TGFB1, SIRT2, RORC, MAPK1, CASP3 |
| 20725      | Nigellimine                                        | ACPH, NOQ1, CYP1A2, GRM4, CCNE2, CDK2, CCNE1, DRYK1A, DRYK1B, JAK1, JAK2, CTSC, GSK3B, CN51KD, MRG5, ADORA2B, IDO1, PDE5A, CDK1, CCNB1, CCNB2 |
| 7439       | Carvone                                            | CYP19A1, SRD5A1, NR3C1, PGR, SERPINA6, SHBG, FABP1, NR1I3, SRD5A2, AR, ADH1A, SIGMARR1, ADH1C, NPC1L1, PTGES, CA2, CYP17A1, PARP1, ADORA3, MAPK3, PRKCH, PTPIN1, AKR1B10, PTPIN2, PTGSI, NR1I3, TOP2A |
| 139038581  | Nigellidine 4-O-sulfite                             | CDK5R1, CDK5, RPS6KA2, MIF |

### TABLE 3 Predicted relationship of Nigella sativa L. target proteins with colorectal cancer Signaling Pathways with STRING database.

| Proteins involved in colorectal cancer | Colorectal cancer signaling pathway | Proteins involved in colorectal cancer signaling pathways |
|---------------------------------------|------------------------------------|----------------------------------------------------------|
| TGFB1                                 | ERK/MAPK signaling pathway          | TGFB1, MAPK3, AKT1, MAPK1, CASP3, EGFR, MAPK8, MAPK10 |
| MAPK3                                 | PI3K-Akt signaling pathway          | MAPK3, AKT1, MAPK1, EGFR, PIK3CA, GSK3B |
| AKT1                                  | WNT signaling pathway               | GSK3B, MAPK8, MAPK10 |
| MAPK8                                 | RAS signaling pathway               | MAPK3, AKT1, MAPK1, EGFR, PIK3CA, MAPK8, MAPK10 |
| MAPK10                                | TGFB signaling pathway              | MAPK1, MAPK3, TGFB1 |
| MAPK1                                 | Apoptotic                            | MAPK3, AKT1, CASP3, MAPK1, PIK3CA, MAPK8, MAPK10 |

According to Carrillo et al. (2012), oleic acid could induce apoptosis in carcinoma cells which can be associated with increased production of intracellular free radical species (ROS) or caspase-3 (CASP3) activity. Based on Salim et al. (2013) research, the results of caspase-3, -8 and -92.
FIGURE 4 Confidence view with STITCH database. The interaction of the metabolite and (a) the main target (surface receptor) protein or (b) the second (intracellular) protein with related activity. Protein-protein interactions are shown in grey, chemical-protein interactions are shown in green and interactions between metabolites are shown in red.

9 activity showed that thymoquinone induces apoptotic. Thymol and CASP3 also offer fairly strong interaction because thymol can cause cellular damage such as lipid degeneration, mitochondrial damage, nucleolar segregation
and apoptotic in intestinal cell lines (Caco-2) (Islam et al. 2019). Myristicin is also predicted to induce apoptotic because it has the same interaction value as thymol–CASP3 (0.700).

Apoptotic is a mechanism that leads to cell death in response to internal and external signals, including the release of cytochrome c from mitochondrial caspase family proteins (CASP). Initiator CASPs (CASP8, -9, and -10) activate effector CASPs (CASP3, -6, and -7), which mediate apoptotic through proteolytic cleavage of thousands of proteins. Various pathways have become therapeutic targets in oncology that induce apoptotic through the apoptotic core pathway, namely the kinase signaling pathway involving AKT, ERK, RAS, RAF, MEK or mTOR. Inhibition of growth factor receptors, including EGFR, HER2, other members of the ErbB family, MET or NTRK, also causes apoptotic (Carneiro and El-Deiry 2020).

The PI3K-AKT signaling pathway is widely activated in all types of cancer known to promote cell growth and survival, inhibit apoptotic, and control metabolism. This pathway is often hyperactivated in cancer due to mutations or deletions in negative regulators. The PI3K pathway is activated by a range of stimuli, including growth factors, such as receptor tyrosine kinases (RTKs) including epidermal growth factor receptors (EGFR) and platelet-derived growth factor receptors (PDGFR) (Slattery et al. 2018; Barata and Oliveira 2019).

Genetic variation in PIK3CA and AKT1 is associated with a strongly increased risk of colon cancer (Slattery et al. 2018). Chromosomal instability in 85% of invasive colorectal cancers accumulates hereditary mutations such as APC, TP53, SMAD4, KRAS, and the catalytic-subunit of PI3K (PIK3CA), which is an adenoma – carcinoma sequence (Vogelstein et al. 2013). The oleic acid in black cumin is predicted to carry a chemical signal received by the surface receptor protein PIK3CA, thereby activating the PI3K-AKT signaling pathway as indicated by an interaction score of 0.900 (Table 4).

AKT (protein kinase B or PKB), a component of intracellular insulin signaling activated by PI3K, is involved in regulating key factors related to cell growth, survival, and proliferation. AKT inhibits glycogen synthase kinase-3α/β, thereby promoting cell viability and proliferation; activates complex I rapamycin (mTORC, consisting of mTORC1 [mTOR, Raptor, mLST8, and PRAS40, Raptor mLST8, and PRAS40] and mTORC2 [mTOR, Rictor, mSin1, and MLST8]) and the ribosomal S6 kinase RPS6KB1 and promotes transcription of the family fork-
head box O (FOXO) proapoptotic protein. During activation, mTORC1 phosphorylates and inactivates eukaryotic initiation factor 4E-binding protein 1 (EIF4BP1) and activates p70 ribosomal S6 kinase (p70S6K), leading to increased protein translation at the ribosome (Slattery et al. 2018; Barata and Oliveira 2019).

In addition to being activated by oleic acid, the PI3K-AKT signaling pathway is also transduced by the TGFβ ligand via the non-canonical TGFβ signaling pathway, MAPK and ROCK signaling pathway pathways. The TGFβ (transforming growth factor-beta) signaling pathway is a very important pathway in colorectal cancer tumorigenesis. In normal cells, this pathway plays a role in suppressing growth and tumorigenesis. Still, cancer cells regulate development, proliferation, differentiation, apoptotic, tissue homeostasis and promote epithelial-mesenchymal transition, invasion, and metastasis (TGFβ paradox) (Jung et al. 2017). In addition to the non-canonical pathway, there is also a canonical pathway involving type 1 BMP and TGFβ receptors, activin, and the substrates SMAD1/5/8, SMAD2/3 (R-SMAD), and SMAD4, which can bind transcription factors as partners in regulating transcription (Hao et al. 2019). TGFβ signaling pathway can also induce epithelial to mesenchymal transition (EMT) in cancer cells leading to poor prognosis and minimal chemotherapy benefits (Okita et al. 2018; Sveen et al. 2018). In this regard, Idrus et al. (2019) revealed that black cumin (Nigella sativa L.) and thymoquinone are associated with EMT, which is regulated by activation of transcription factors, promoting wound healing, and reducing the reduction of wound healing tissue inflammation, and preventing organ fibrosis through regulation of the EMT process.

The MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathway transduced by the non-canonical TGFβ pathway is at the core of a signaling network involved in regulating cell growth, development and division (Kovetitypour et al. 2019). Most of the target proteins of black cumin metabolites are associated with the MAPK/ERK signaling pathway in colorectal cancer through surface receptor proteins MAPK1, -3, -8, and -10, TGFBR1, AKT1, EGFR, and CASP3. Guo et al. (2020) stated that the ERK1/2 signaling pathway is involved in cell survival after intestinal injury, and inhibition of this pathway may promote apoptotic of gut-injured cells.

In addition to the TGFβ signaling pathway, the MAPK/ERK signaling pathway is activated by the Ras signaling pathway involving RAF–MEK–ERK/MAPK, which transmits signals downstream and results in the transcription of genes involved in controlling several cellular mechanisms. Ras communicates external cellular signals to the nucleus, and its altered activation leads to inappropriate cellular activities, including increased cell growth, differentiation, survival, and cancer. Ras activation allows interaction with several downstream effectors or intracellular proteins (MAPK3, AKT1, MAPK1, EGFR, PIK3CA, MAPK8, MAPK10) (Santarpia et al. 2012).

Another colorectal cancer signaling pathway is Wnt, in which one of the major regulators or surface receptor proteins involved is GSK-3 which is also involved in the PI3K-AKT signaling pathway. Increased levels of GSK-3 present demonstrate the tumour-promoting action of GSK-3 in certain tumour types and/or by the antiproliferative effect of GSK-3 inhibitors, such as in colon and pancreatic cancers (Tejeda-Muñoz and Robles-Flores 2015).

Our research provides a pharmacological network prediction of black cumin (Nigella sativa L.) as a candidate for OMAI in colorectal cancer through an in silico test. Our results support Hsu et al. (2017), which stated that high doses of thymoquinone showed antiproliferative effects on human colonic adenocarcinoma LoVo cells through downregulation of downregulation of p–PI3K, p-Akt, p-GSK3B and -catenin, as well as COX-2 and prostaglandin E2 (PGE2). Thymoquinone also showed efficacy and promoted autophagy and apoptotic in irinotecan-resistant LoVo colon cancer cells (CPOI-11-R) by inducing mitochondrial outer membrane permeability, activating JNK and p38, and inhibiting the NF-κB, ERK1/2, and PI3K pathways (Chen et al. 2017). Increased phosphorylation of mitogen-activated protein kinase (MAP) p38 (Woo et al. 2013), inhibition of MEK–ERK1/2 signaling (El-Baba et al. 2014), and blocking of the PI3K-Akt signaling pathway are also other mechanisms of thymoquinone as anticancer (Dirican et al. 2015). On the other hand, thymoquinone also has interactions with other metabolites also contained in black cumin, namely thymol, nigellimine, and p-cymene. This proves that plant-based treatment with a multicomponent approach allows synergistic interactions between active compounds that support the resulting pharmacological effects (Syahrir et al. 2016).

Some of the advantages of the in silico method are reducing the number of compounds or molecules and the increased speed of research of most compound and protein interactions through database searches and reducing the use of experimental animals in predictive studies. This in silico prediction can also save time on developing ligand-based drugs and can be a starting point before conducting in vitro and in vivo tests, as well as proving the opportunity for black cumin to become a candidate for OMAI. However, the weakness of this method also requires consideration, including a large amount of data analyzed. It requires a high level of accuracy and has the risk of bias depending on the database used to predict. Some of the active metabolites in black cumin that are not in the database

The MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathway transduced by the non-canonical TGFβ pathway is at the core of a signaling network involved in regulating cell growth, development and division (Kovetitypour et al. 2019). Most of the target proteins of black cumin metabolites are associated with the MAPK/ERK signaling pathway in colorectal cancer through surface receptor proteins MAPK1, -3, -8, and -10, TGFBR1, AKT1, EGFR, and CASP3. Guo et al. (2020) stated that the ERK1/2 signaling pathway is involved in cell survival after intestinal injury, and inhibition of this pathway may promote apoptotic of gut-injured cells.

In addition to the TGFβ signaling pathway, the MAPK/ERK signaling pathway is activated by the Ras signaling pathway involving RAF–MEK–ERK/MAPK, which transmits signals downstream and results in the transcription of genes involved in controlling several cellular mechanisms. Ras communicates external cellular signals to the nucleus, and its altered activation leads to inappropriate cellular activities, including increased cell growth, differentiation, survival, and cancer. Ras activation allows interaction with several downstream effectors or intracellular proteins (MAPK3, AKT1, MAPK1, EGFR, PIK3CA, MAPK8, MAPK10) (Santarpia et al. 2012).

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### TABLE 4 Interaction analysis of target proteins and metabolites in Nigella sativa L. with STITCH database.

| Node 1        | Node 2 | Score |
|---------------|--------|-------|
| thymoquinone  | CASP3  | 0.822 |
| thymol        | CASP3  | 0.700 |
| oleic acid    | PIK3CA | 0.800 |
| oleic acid    | CASP3  | 0.828 |
| myristicine   | CASP3  | 0.700 |
can also increase the chance of error in predictions and reduce the accuracy of research results.

4. Conclusions

Black cumin (Nigella sativa L.) has a pharmacology network that describes the correlation between the metabolites contained there, targets or surface receptor and intracellular proteins, and colorectal cancer signaling pathways. This indicates that black cumin (Nigella sativa L.) has the potential as OMAI and can be a reference in the development of cancer treatment, especially colorectal cancer.

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Authors’ contributions

SR, FN, SB, SS designed the study. SR, FN, SB, SS, AF analyzed the data. SR, FN, SB, SS, AF wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The author declare that they have no competing interest.

References

Altman T, Travers M, Kothari A, Caspi R, Karp PD. 2013. A systematic comparison of the MetaCyc and KEGG pathway databases. BMC Bioinformatics 14(112):1–15. doi:10.1186/1471-2105-14-112.

Barata JT, Oliveira ML. 2019. Cell Signaling in Cancer. In: R Fior, R Zilhão, editors, Molecular and Cell Biology of Cancer: When Cells Break the Rules and Hijack Their Own Planet, chapter 3. Switzerland: Springer Nature Switzerland AG, 1 edition. p. 31–43. doi:10.1007/978-3-030-11812-9_3.

Carneiro BA, El­Deiry WS. 2020. Targeting apoptosis in cancer therapy. Nat. Rev. Clin. Oncol. 17(2):145–151. doi:10.1038/s41561-020-0341-y.

Carrillo C, Cavia MDM, Alonso­Torre SR. 2012. Antitumor effect of oleic acid; mechanisms of action: a review. Nutr. Hosp. 27(5):1860–1865. doi:10.3305/nh.2012.27.6090.

Chen M, Lee N, Hsu H, Ho T, Tu C, Chen R, Yueh­Min Lin Y, Viswanadha V, Kuo W, Huang C. 2017. Inhibition of NF­B and metastasis in irinotecan (CPT­11)­resistant LoVo colon cancer cells by thymoquinone via JNK and p38. Environ. Toxicol. 32(2):669–678. doi:10.1002/tox.22268.

Corso M, Perreau F, Mouille G, Lepiniec L. 2020. Specialized phenolic compounds in seeds: structures, functions, and regulations. Plant Sci. 296(110471):1–15. doi:10.1016/j.plantsci.2020.110471.

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

Dirican A, Atmaca H, Bozkurt E, Erten C, Karaca B, Uslu R. 2015. Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3KAKT pathway. Clin. Transl. Oncol. 17(2):145–151. doi:10.1007/s12094­014-1206-6.

Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. 2019. Cytoscape StringApp: Network analysis and visualization of proteomics data. J. Proteome Res. 18(2):623–632. doi:10.1021/acs.jproteome.8b00702.

El­Baba C, Mahadevan V, Fahibusch F, Mohan S, Rau T, Gali­Muhtasib H, Schneider­Stock R. 2014. Thymoquinone­induced conformational changes of PAK1 interrupt prosurvival MEK­ERK signaling in colorectal cancer. Mol. Cancer 13:201. doi:10.1186/1476-4598-13-201.

Filimonov DA, Lagunin AA, Gloriosoza TA, Rudik AV, Druzhilovskii DS, Pogodin PV, Poroikov VV. 2014. Prediction of the biological activity spectra of organic compounds using the pass online web resource. Chem. Heterocycl. Compounds 50(3):444–457. doi:10.1007/s10593-014-1496-1.

Florescu­Ţenea RM, Kamal AM, Mitruţ P, Mitruţ R, Ilie DS, Nicolaescu AC, Mogoanţă L. 2019. Colorectal Cancer: An update on treatment options and future perspectives. Curr. Health Sci. J. 45(2):134–141. doi:10.12865/CHSJ.45.02.02.

Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. 2014. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 42(W1):W32–W38. doi:10.1093/nar/gku293.

Guney E, Menche J, Vidal M, Barabasi A. 2016. Network-based in silico drug efficacy screening. Nat. Commun. 7(1):1–13. doi:10.1038/ncomms10331.

Guo Y, Pan W, Liu S, Shen Z, Xu Y, Hu L. 2020. ERK/MAPK signalling pathway and tumorigenesis. Exp. Ther. Med. 19(3):1997–2007. doi:10.3892/etm.2020.8454.

Hao Y, Baker D, Ten Dijke P. 2019. TGF-β-mediated epithelial-mesenchymal transition and cancer metastasis. Int. J. Mol. Sci. 20(11):1–34. doi:10.3390/ijms20112767.

Hosseinzadeh H, Mollazadeh H, Ashari A. 2017. Review on the potential therapeutic roles of Nigella sativa in the treatment of patients with cancer: Involvement of apoptosis. J. Pharmacopuncture 20(3):158–172. doi:10.3831/kpi.2017.20.019.

Hsu H, Chen M, Day C, Lin Y, Li S, Tu C, Padma V,
Shih H, Kuo W, Huang C. 2017. Thymoquinone suppresses migration of LoVo human colon cancer cells by reducing prostaglandin E2 induced COX-2 activation. World J. Gastroenterol. 23(7):71171–71179. doi:10.3734/wg.v23i7.1171.

Idrus R, Nordin A, Kamal H, Yazid M, Saim A. 2019. Effect of Nigella sativa and its bioactive compound on type 2 epithelial to mesenchymal transition: a systematic review. BMC Complementary Altern. Med. 19(290):1–12. doi:10.1186/s12906-019-02076-2.

Islam M, Khalhipa A, Bagchi R, Mondal M, Smrity S, Uddin S, Shilpi J, Rouf R. 2019. Anticancer activity of Thymol: A literature-based review and docking study with Emphasis on its anticancer mechanisms. IUBMB Life 71(1):9–19. doi:10.1002/iub.1935.

Juan C, Agahi F, Font G, Juan-Garcia A. 2020. In silico methods for metabolomics and toxicology prediction of zearalenone, α-zearalenone, and β-zearalenone. Food Chem. Toxicol. 146:1–10. doi:10.1016/j.fct.2020.111818.

Jung B, Staudacher J, Beauchamp D. 2017. Transforming Growth Factor beta Superfamily Signaling in Development of Colorectal Cancer. Gastroenterology 152(1):36–52. doi:10.1053/j.gastro.2016.10.015.

Khan MA, Tania M, Fu S, Fu J. 2017. Thymoquinone, as an anticancer molecule: from basic research to clinical investigation. Oncotarget 8(31):51907–51919. doi:10.18632/oncotarget.17206.

Kononenko O, Baysal O, Holmes R, Godfrey MW. 2014. Mining modern repositories with Elasticsearch. In: Proceedings of the 11th Working Conference on Mining Software Repositories, MSR 2014. New York, NY, USA: Association for Computing Machinery. p. 328–331. doi:10.1145/2597073.2597091.

Kooi W, Hasanzadeh-Noohi Z, Sharafi-Alvazni N, Asadisamani M, Ashtary-Larky D. 2016. Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). Chin. J. Nat. Med. 14(10):732–745. doi:10.1016/s1875-5364(16)30088-7.

Koveitpour Z, Panahi F, Vakilian M, Peymani M, Forootan F, Esfahani M, Ghaedi K. 2019. Signaling pathways involved in colorectal cancer progression. Cell Biosci. 9:1–14. doi:10.1186/s13578-019-0361-4.

Li M, Li D, Tang Y, Wu F, Wang J. 2017. CytoCluster: A cytoscope plugin for cluster analysis and visualization of biological networks. Int. J. Mol. Sci. 18(9):1–13. doi:10.3390/ijms18091880.

Ministry of Health of the Republic of Indonesia. 2018. Pedoman Nasional Pelayanan Kedokteran Tata Laksana Kanker Kolerakal. Jakarta: Ministry of Health of the Republic of Indonesia.

National Agency of Drug and Food Control of the Republic of Indonesia. 2020. Informatorium Obat Modern Asli Indonesia (OMAI) di Masa Pandemi COVID-19. Jakarta: National Agency of Drug and Food Control of the Republic of Indonesia.

Okita A, Takahashi S, Ouchi K, Inoue M, Watanabe M, Endo M, Honda H, Yamada Y, Ishioka C. 2018. Consensus molecular subtypes classification of colorectal cancer as a predictive factor for chemotherapeutic efficacy against metastatic colorectal cancer. Oncotarget 9(27):18698–18711. doi:10.18632/oncotarget.24617.

Recio-Boilés A, Cagir B. 2021. Colon Cancer. StatPearls Publishing. URL https://www.ncbi.nlm.nih.gov/books/NBK470380/.

Salim L, Mohan S, Othman R, Abdelwahab S, Kalimadeghan B, Sheikh B, Ibrahim M. 2013. Thymoquinone induces mitochondria-mediated apoptosis in acute lymphoblastic leukaemia in vitro. Molecules 18(9):11219–11240. doi:10.3390/molecules180911219.

Santralia P, Lippman SM, El-Naggar AK. 2012. Targeting the MAPK–RAS–RAF signaling pathway in cancer therapy. Expert Opin. Ther. Targets 16(1):103–119. doi:10.1517/14728222.2011.645805.

Savithramma N, Yungandhar P, Prasad KS, Ankanna S, Chetty KM. 2016. Ethnomedicinal studies on plants used by Yanadi tribe of Chandragiri reserve forest area, Chittoor District, Andhra Pradesh, India. J. Intercult. Ethnopharmacol. 5(1):49–56. doi:10.5455/jice.20160122065531.

Singer S. 2018. Psychosocial impact of cancer. Recent Results Cancer Res. 210(210):1–11. doi:10.1007/978-3-642-43106-6_1.

Slattery M, Mullany L, Sakoda L, Wolff R, Stevens J, Samowitz W, Herrick J. 2018. The PI3K/AKT signaling pathway: Associations of miRNAs with dysregulated gene expression in colorectal cancer. Mol. Carcinog. 57(2):243–261. doi:10.1002/mc.22752.

Sutiono R, Salim MMGR, Nadya, Liani O, Susanto S. 2017. Native Indonesian Herbs: Challenges in The Future for Anti-Cancer Drugs. Cermin Dunia Kedokteran 44(11):822–826. doi:10.55175/cdk.v44i11.708.

Sween A, Bruun J, Eide P, Vellertsen I, Ramirez L, Murumägi A, Arjama M, Danielsen S, Kryeziu K, Elez E, Tabenero J, Guinney J, Palmer H, Nesbakken A, Kallioniemi O, Dienstmann R, RA L. 2018. Colorectal cancer consensus molecular subtypes translated to preclinical models uncover potentially targetable cancer cell dependencies. Clin. Cancer Res. 24(4):794–806. doi:10.1158/1078-0432.CCR-17-1234.

Syahrir NHA, Afendi FM, Susetyo B. 2016. Efek sinergis bahan aktif tanaman obat basiskan jejaring dengan protein target. Jurnal Jamu Indonesia 1(1):35–46. URL http://biofarmaka.ipb.ac.id/biofarmaka/2017/Jurnal%20Jamu%20Indonesia%20Vol%201%20No%201%20Artikel%20025.pdf.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering Cv. 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res.
Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. 2016. STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 44(D1):D380–4. doi:10.1093/nar/gkv1277.

Tejeda-Muñoz N, Robles-Flores M. 2015. Glycogen synthase kinase 3 in Wnt signaling pathway and cancer. IUBMB Life 67(12):914–922. doi:10.1002/iub.1454.

Vogelstein B, Papadopoulos N, Velculescu V, Zhou S, Diaz, LA J, Kinzler K. 2013. Cancer genome landscapes. Science 339(6127):1546–1558. doi:10.1126/science.1235122.

Woo C, Hsu A, Kumar A, Sethi G, Tan K. 2013. Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: the role of p38 MAPK and ROS. PLoS One 8(10):e75356. doi:10.1371/journal.pone.0075356.

Yi F, Liu H, Li L, Xu L, Meng H, Dong Y, Xiao P. 2018. In silico approach in reveal traditional medicine plants pharmacological material basis. Chinese Med. CHIN MED-UK 13(1):1–20. doi:10.1186/s13020-018-0190-0.