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Original Article

Ethnopharmacological study of flavonoid compounds in *Magnolia champaca* (L.) Baill. ex Pierre as anti-inflammatory agents by molecular docking

[Estudio etnofarmacológico de compuestos flavonoides en *Magnolia champaca* (L.) Baill. ex Pierre como agentes antiinflamatorios por acoplamiento molecular]

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Abstract

Context: *Magnolia champaca* (L.) Baill. ex Pierre has traditionally been used by the culture to prevent and cure the inflammatory disease.

Aims: To determine the benefit of *M. champaca* in the local community, especially in the treatment of tuberculosis, and investigate the potency of the flavonoid content of *M. champaca* as an anti-inflammatory agent through *in silico* analysis.

Methods: An ethnobotanical survey was conducted by structured interviews and responses in the Pamekasan district. The identification of flavonoid in selected plant was carried out from literature. Then, quercetin, (-)-epicatechin, and kaempferol were docked with protein targets including cyclooxygenase-2 (COX-2), mitogen-activated protein kinases (p38 MAPK), nuclear factor kappa B (NF-κB), and phosphoinositide 3 kinases (PI3k). The ability of complex compounds was considered dependent on energy binding and the ability to bind native ligand to proteins.

Results: *M. champaca* exhibited the highest RFI value, indicated that this plant mainly used to treat tuberculosis symptoms in the local community. The compounds of quercetin and (-)-epicatechin can only be bound to a native ligand COX-2, NAG. The compounds quercetin, rutin, kaempferol, and (-)-epicatechin can then be bound to both the native proteins NF-κB and PI3K. Nevertheless, native ligand-protein p38 MAP-kinases cannot be bound by complex compounds like quercetin, rutin, kaempferol, and (-)-epicatechin.

Conclusions: The research offers proof for considering the flavonoid compound in *M. champaca* as a beneficial ligand complex throughout the treatment and prevention of inflammatory diseases. Further *in vitro* and *in vivo* studies could prove its therapeutic potential.

Keywords: anti-inflammatory candidate; ethnopharmacological study; *Magnolia champaca*; molecular docking.

Resumen

Contexto: *Magnolia champaca* (L.) Baill. ex Pierre ha sido utilizado tradicionalmente por la cultura para prevenir y curar enfermedades inflamatorias.

Objetivos: Determinar el beneficio de *M. champaca* en la comunidad local, especialmente en el tratamiento de la tuberculosis, e investigar la potencia del contenido de flavonoides de *M. champaca* como agente antiinflamatorio mediante análisis *in silico*.

Métodos: Se realizó una encuesta etnobotánica mediante entrevistas estructuradas y respuestas en el distrito de Pamekasan. La identificación de flavonoides en plantas seleccionadas se realizó a partir de la literatura. Luego, quercetina, (-) - epicatequina y kaempferol se acoplaron a proteínas dianas que incluían ciclooxigenasa-2 (COX-2), proteína quinasas activadas por mitógenos (p38 MAPK), factor nuclear *kappa* B (NF-κB) y fosfoinositida 3. quinasas (PI3k). Se consideró que la capacidad de los compuestos complejos dependía de la unión de energía y la capacidad de unir ligando nativo a proteínas.

Resultados: *M. champaca* exhibió el valor de RFI más alto, indicando que esta planta se usa principalmente para tratar síntomas de tuberculosis en la comunidad local. Los compuestos de quercetina y (-)-epicatequina solo pueden unirse a un ligando nativo COX-2, NAG. Los compuestos quercetina, rutina, kaempferol y (-)-epicatequina pueden unirse a las proteínas nativas NF-κB y PI3K. Sin embargo, las MAP-quinasas p38 ligando-proteína nativas no pueden unirse a compuestos complejos como quercetina, rutina, kaempferol y (-)-epicatequina.

Conclusiones: La investigación ofrece pruebas para considerar el compuesto flavonoide de *M. champaca* como un complejo ligando beneficioso en el tratamiento y prevención de enfermedades inflamatorias. Otros estudios *in vitro* e *in vivo* podrían demostrar su potencial terapéutico.

Palabras Clave: acoplamiento molecular; candidato antiinflamatorio; estudio etnofarmacológico; *Magnolia champaca*.

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INTRODUCTION

The development of traditional medicine is limited to the interests of culture, biodiversity and health maintenance. Local crops can be used as herbal medicine to prevent and treat disease. Traditional plant utilization is a secure method, as the technique has been empirically proved by the local community (Cheikhyoussef et al., 2011). The local people traditionally use the *Magnolia champaca* as a major option in preventing and treating diseases (Ahmad et al., 2011). Plant parts, such as flowers and leaves, can act as anti-inflammatory agents (Ananthi and Anuradha, 2015). The content of flavonoids in plants can affect proinflammatory activities by decreasing IL-6 and tumor necrosis factor (TNF-α) cytokines (O'Garra et al., 2013; Liu et al., 2017; Pompermaier et al., 2018). The cytokine is activated by macrophage and then induce the expression of nuclear factor *kappa* B (NF-κB) and cyclooxygenase-2 (COX-2) inflammatory mediators in the innate immune system. The innate immune system response involves macrophage activation that causes T cell differentiation (Lee et al., 2011; Liu et al., 2017).

Mycobacterium tuberculosis infections are followed by local inflammatory reactions as the initial tuberculosis pathogenesis (O'Garra et al., 2013). The inflammatory response activates the innate immune by recruitment the polymorphonuclear phagocytes (PMN) and mononuclear phagocytes and then induce the proinflammatory cytokine TNF-α (Parente et al., 2013). The presence of an innate immune response plays an essential role in the initial response of *M. tuberculosis* infections, especially when the bacteria are present in granulomas (O'Garra et al., 2013). *M. tuberculosis* has a potent protein, and non-protein component, which can induce cytokines and chemokines in PMN and monocytes. $CD4^+$ T cells secrete IL-12, IFN-γ, and TNF-α, which are essential to control *M. tuberculosis* infections (O'Garra et al., 2013). The activity of p38 MAP-kinases influences the activation of cytokines as IL-12 and TNF-α in macrophages. Besides, IL-10 cytokine inhibits TNF-α activation by inhibiting p38 kinases (Dong et al., 2002). Recent evidence indicates that p38 MAP-kinases (p38 MAPK) cause pathogenic-induced necrosis (Parente et al., 2013) and mitochondrial membrane disorders (Gräb and Rybniker, 2019). The adaptive immune system is mainly played out by B cells. The production of phosphoinositide 3 kinases (PI3K) is very critical for the activation of BCR signaling (O'Garra et al., 2013). Other studies show that the regulation of BCR is affected by p38 MAP-kinases and NF-κB (Dal Porto et al., 2004).

Inflammatory protein mediators have inhibitory and activation properties that can be understood through molecular docking (Miladiyah et al., 2017). The proteins interact with the ligand active compounds of natural substances (Heeba et al., 2012). Some active compounds are used for molecular docking studies (Yunta, 2016). This active compound is a group of flavonoids found in *M. champaca* (Ahmad et al., 2011). Some active compounds in plants, including rutin, quercetin, (-)-epicatechin, and kaempferol, are active ligands that may bind the target proteins by molecular docking analysis. Virtual screening, shape, and pharmacophore research, and molecular fusion have been used to select chemicals targeting specific proteins or enzymes (Herli et al., 2016). The identification of potential protein targets can be used to determine the characteristics of the best phytochemical components. It is necessary for the treatment or prevention of diseases (Chen et al., 2012). Nowadays, herbal medicine such as *jamu* (a local name of traditional herbal medicine in Indonesia that commonly consists of a mixture of several plant extract) has been usually used to treat diseases (Elfahmi et al., 2014). Therefore, the analysis of molecular docking compounds in plants involved in the production of *jamu* becomes very important. The molecular docking of several active compounds *M. champaca* is very interesting to know the effect of the plant's active compounds on inflammatory mechanisms. The target proteins in this study were COX-2, p38 MAPK, NF-κB, and PI3k. The binding energy of proteins against ligand copying and native ligand may affect these compounds' potential to bind to proteins, native ligand, and ligand copying.

Several studies focus on the molecular docking of a single compound in *M. champaca* (Sinha and Varma, 2016). Our study focused on the flavonoid compound group contained in *M. champaca,* which is expected to have anti-inflammatory activity through molecular docking analysis. All selected compounds were docked together to a target protein to obtain the complex compound's maximal effectivity. Several target proteins were selected based on their potency as an anti-inflammatory agent. The study's findings could provide information about the mechanism of *M. champaca* as an inflammatory agent based on *in silico* analysis, which the plant is commonly used in *jamu* treatment for treating tuberculosis (Elfahmi et al., 2014). This study used the combination of several compounds in *M. champaca* to determine each compound's potency to bind with the native ligand of target protein through the same amino acid residues. The more copy of ligands with the same amino acid residue and native ligand, the compound will easily bind to the native ligand (Chen et al., 2012; Yunta, 2016).

Drug resistance due to the treatment process is often occurred, especially in diseases with *Mycobacterium tuberculosis* infection. Several therapies for this disease have been developed, but the bacteria possessed self-defense and lead to the drug resistant. In the Pamekasan district, local people commonly used *Magnolia champaca* to treat and prevent tuberculosis symptoms. However, there are no studies regarding the effectiveness of this herb to treat tuberculosis. The study expected that a combination of ethnobotany and molecular docking studies could provide preliminary data regarding the effectiveness of *M. champaca* to treat tuberculosis.

Magnolia champaca (L.) Baill. ex Pierre (*Magnoliaceae*) has been known as traditional herbal medicine to treat tuberculosis in the local community (Ahmad et al., 2011). However, limited studies were investigating the potency of *M. champaca* compound as an anti-inflammatory agent. In this study, *M. champaca* compounds were combined and then docked together with several anti-inflammatory proteins *in silico* analysis. Therefore, this study aimed to determine the benefit of *M. champaca* in the local community, especially in the treatment of tuberculosis, and investigate the potency of the flavonoid content of *M. champaca* as an anti-inflammatory agent through *in silico* analysis.

MATERIAL AND METHODS

Ethnobotanical survey

This research was conducted in Pagantenan District, Pamekasan Madura Regency for three weeks. The research tools include questionnaires, books for the identification of medicinal plants, stationery, and Global Positioning System (7°6'29.0586"S and 113°28'32.2572''E). The research object is the respondents of herbal medicine makers, which choose randomly at Pagantenan District. Data are the names and techniques for gathering medicinal plants. The data was obtained through free or structured interviews, specifically about using medicinal plants to prevent tuberculosis (TB) symptoms. Besides, a literature study was conducted from books and scientific journals.

An ethnobotanical survey was conducted between April 2020 and July 2020 to determine the therapeutic values of plant species used to treat TB symptoms in the Pamekasan district. The standard ethnobotanical procedures were followed. An observation technique on cultural history is based on interviews and literature studies from books or other references. Data were collected based on structured interviews and responses recorded by the researcher. Interviews were performed in the local language of the participants because most of them did not formally educate. All interviews were performed after obtaining the verbal prior informed consent from participants (Heinrich et al., 2009).

Protein selection and preparation

PDB structure of molecules COX-2 (pdb id: 5F19), p38 MAP-kinases (pdb id: 6m95), NF-κB (pdb id: 4f3i) and PI3K (pdb id: 6BTY) were retrieved from RCSB Protein Data Bank (PDB) (http://www.rcsb.org/). The protein was prepared for molecular docking by removal of water

molecules, cofactors, and addition of charges and hydrogen atoms using Discovery Studio (DS).

Ligand preparation

(-)-Epicatechin (PubChem id: 72276), rutin (PubChem id: 5280805), quercetin (PubChem id: 5280343), and kaempferol (PubChem id: 5280863) were active compounds of flavonoid used as ligand. The active compound's four structures were downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov) in file format pdb.

Virtual screening and molecular docking analysis

AutoDock4.2.6 software suite was used as a molecular docking tool to carry out the docking simulations (Morris et al., 2009). Pre-calculated grid maps were obtained using Auto Grid, which stores grids of interaction energy based on the ligand atom probes' interaction with the receptor target. The similarity of the docked structures was calculated by measuring the root mean square deviations between the atoms' positions and the construction of a cluster of conformations. The RMSD values and the lowest binding energy conformation in all clusters were considered the most suitable docking pose. However, this research only used the highest binding energy. Then, docking logs were analyzed in the graphical user interface of Auto Dock Tools (ADT), and Python scripts in the MGL tools package were used to analyze the docking results (Hess et al., 2008). The outputs from Auto Dock studies as well as images were generated with PyMol (Sanner, 1999).

Data analysis

Descriptive analysis with a qualitative approach was used to analyze the ethnobotanical data. The interviews' data were analyzed using the Relative Frequency Index (RFI) to determine the most influential species in treating TB symptoms and TB disease (de Santana et al., 2016). The RFI is an index of the local importance of each species. The RFI values were measured using the following equation [1] (Constant and Tshisikhawe, 2018):

$$RFI = FC/N \quad [1]$$

where, FC: number of respondents that mention the use of a species and N: total number of the respondents.

RESULTS AND DISCUSSION

Ethnobotanical studies

According to survey results, there were 12 plants used in the local community to treat several diseases (Table 1). From these plants, 8 plants were used to treat other diseases except tuberculosis, including *Curcuma amanda* Roxb., *Zingiberaceae* (12%), *Urena lobata* L., *Malvaceae* (4%), *Barleria prionitis* L., *Acanthaceae* (4%), *Abelmoschus moschatus* Medik., *Malvaceae* (4%), *Centella asiatica* (L.) Urb., *Apiaceae* (4%), *Ocimum* × *africanum* Lour., *Lamiaceae* (8%), *Boesenbergia rotunda* (L.) Mansf., *Zingiberaceae* (8%), and *Jatropha gossypiifolia* L., *Euphorbiaceae* (4%). While four plants were used to treat tuberculosis, such as *Magnolia champaca* (L.) Baill. ex Pierre. *Magnoliaceae* (20%), *Magnolia* × *alba* (DC.) Figlar, *Magnoliaceae* (12%), *Piper retrofractum* Vahl, *Piperaceae* (16%), and *Alstonia scholaris* (L.) R. Br., *Apocynaceae* (4%). *Ricinus communis* L. (*Euphorbiaceae*) was found in survey location, but the local community did not use it for disease treatment.

Based on the results of studies in local communities, it is known that some of the plants used in the treatment of TB symptoms. Ethnobotany research used to treat TB has also been carried out by other countries such as Laos (Elkington et al., 2009). The community has used some plants from the family *Rutaceae*, *Euphorbiaceae*, and *Apocynaceae* to treat TB (Elkington et al., 2014). Based on Table 1, three families of plants have been used by local communities to treat several diseases, especially for the health of mothers and children. It shows the maintaining Madurese community's cultural characteristics applying medicinal plant starting from the family environment.

Table 1. Medicinal plants used to treat diseases and Relative Frequency Index (RFI).

Local Name	Family	Species	Use of plant part	RFI (%)
Cabe jamu	*Piperaceae*	*Piper retrofractum* Vahl.	Fruit	16
Cempaka putih	*Magnoliaceae*	*Magnolia alba* (DC.) Figlar	Flower, root, leaf	12
Cempaka kuning	*Magnoliaceae*	*Magnolia champaca* (L.) Baill. ex Pierre.	Flower, root, leaf	20
Jarak ulung	*Euphorbiaceae*	*Jatropha gossypiifolia* L.	Leaf	4
Temu kunci	*Zingiberaceae*	*Boesenbergia rotunda* (L.) Mansf.	Rhizome	8
Kemangi	*Lamiaceae*	*Ocimum africanum* Lour.	Leaf	8
Kaki kuda	*Apiaceae*	*Centella asiatica* (L.) Urb.	All of the plant parts	4
Kapasan	*Malvaceae*	*Abelmoschus moschatus* Medik.	Root, leaf, seed and flower	4
Landep	*Acanthaceae*	*Barleria prionitis* L.	Leaf and root	4
Pulai	*Apocynaceae*	*Alstonia scholaris* (L.) R. Br.	Tree bark and leaf	4
Pulutan	*Malvaceae*	*Urena lobata* L.	All of the plant parts	4
Temu mangga	*Zingiberaceae*	*Curcuma amada* Roxb.	Rhizome	12
Jarak wulung	*Euphorbiaceae*	*Ricinus communis* L.	Leaf	0

The RFI value was obtained from all medicinal plants, which were used to treat all disease (tuberculosis and non-tuberculosis), obtained from 25-selected respondent.

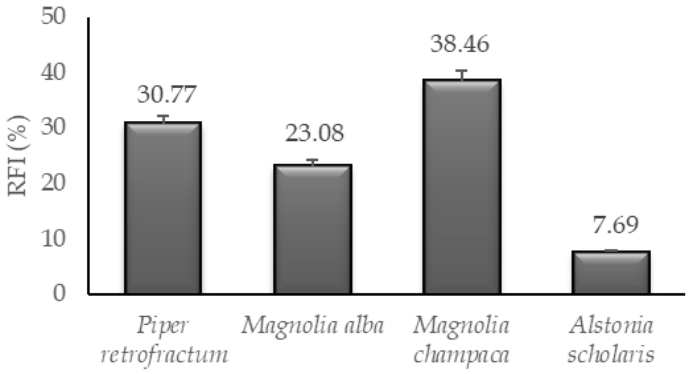

Figure 1. Results of Relative Frequency Index (RFI) analysis of potential plants to treat tuberculosis symptoms.

The RFI value was obtained from 13 respondents from a total of 25-selected respondents. These 13 respondents declared to use *Piper retrofractum*, *Magnolia alba*, *Magnolia champaca* and *Alstonia scholaris* to treat tuberculosis symptoms.

The RFI calculation (Fig. 1) was performed to determine which species mainly used to prevent TB disease symptoms in the local community. *Magnolia champaca* plants possessed the highest RFI average (38.46%), followed by *Piper retrofractum* (30.77%). Based on studies in local communities, these plants were the primary commodities of the Pamekasan district used for mother and child health. Based on Fig.1, *Magnolia alba* showed a RFI value of 23.08%. The community uses these plants to treat TB symptoms, but there are no supporting references related to its plants' bioactivity in treating tuberculosis. Furthermore, *Alstonia scholaris* exhibited the lowest RFI of 7.69%.

Age range and educational background of the respondents

Based on Fig. 2, 40% of respondents aged 35-44 years had different backgrounds in utilizing medicinal plants to prevent and treat tuberculosis. Meanwhile, 24% of respondents aged 55-64, 16% of respondents aged 45-54 and 65-74 years, and 4% of respondents aged more than 75 years.

Most respondents have a high school education background (36%) and followed by junior high school (28%), primary school (24%), and undergraduate graduates (12%) (Fig. 3). The selected respondents exhibited experience using medicinal

plant from the previous generation and passed on to the next generation. Experience gained during their education, especially at bachelor's degree was making herbal medicine and marketing it.

Based on Fig. 3, 38% of respondents aged 35-44 years declared experience using medicinal plants for treating tuberculosis symptoms. Furthermore, 23% of respondents aged 55-64 years, 15% of respondents aged 45-54 and 65-74 years, and 8% of respondents aged over 75 years, had experience using plants for tuberculosis symptoms. While respondents used the non-tuberculosis symptom plants with an age range of 35-44 years (42%), 55-64 (25%), 45-54, and 65-74 years (17%) and over 75 years (0%).

The people who use plants for TB symptoms have the highest educational background at elementary school and junior high school (31%) (Fig. 4), followed by senior high school (23%) and bachelor's degree (15%). Furthermore, the people who use plants for non-tuberculosis symptoms were a senior high school background (50%), then followed by junior high school (25%), elementary school (17%), and bachelor's degree (8%).

Most of the respondents aged 35-44 years have a high school senior education and bachelor's degree background. The utilization of medicinal plants in various ages and educational backgrounds mostly comes from the previous generation. In that generation, they have experience treating and preventing non-tuberculosis disease, such as fitness and body care. Then, respondents with background bachelor's degrees choose medicinal plants for TB based on their knowledge during their education. Respondents in the age range of 45-54 years, some have a background in senior high school and junior high school education. Therefore, the use of medicinal plants is passed down from the previous generation. The respondents aged 55-64 and senior high school background had experience using medicinal plants based on information from the previous generation. Some others in junior high school and elementary school have experience in formulating and marketing herbal medicine. Respondents in the age range of 65-74 have senior high school and elementary school education backgrounds who have experience in treatment based on previous generations' information, formulating, and marketing herbal medicine. Furthermore, one respondent (aged ≥75 years) with a background in elementary school has experience in herbal medicine's formulation and marketing.

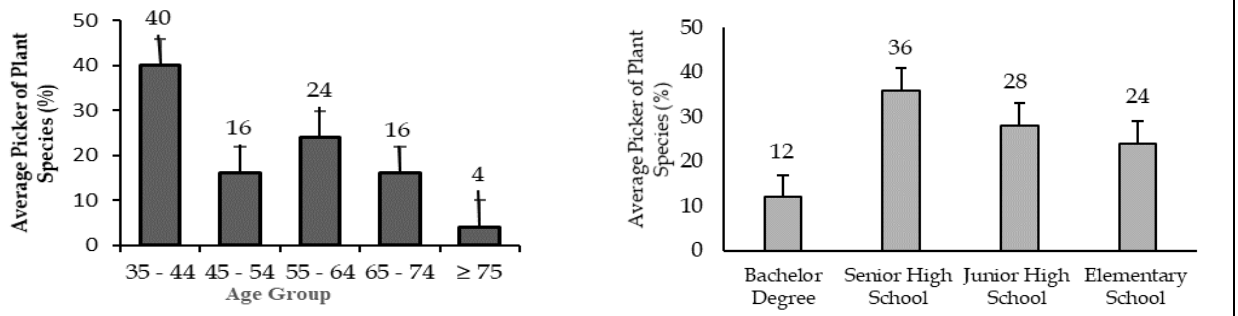

Figure 2. The average picker of plant species according to **(A)** age range of respondents and **(B)** the educational background of the respondents.

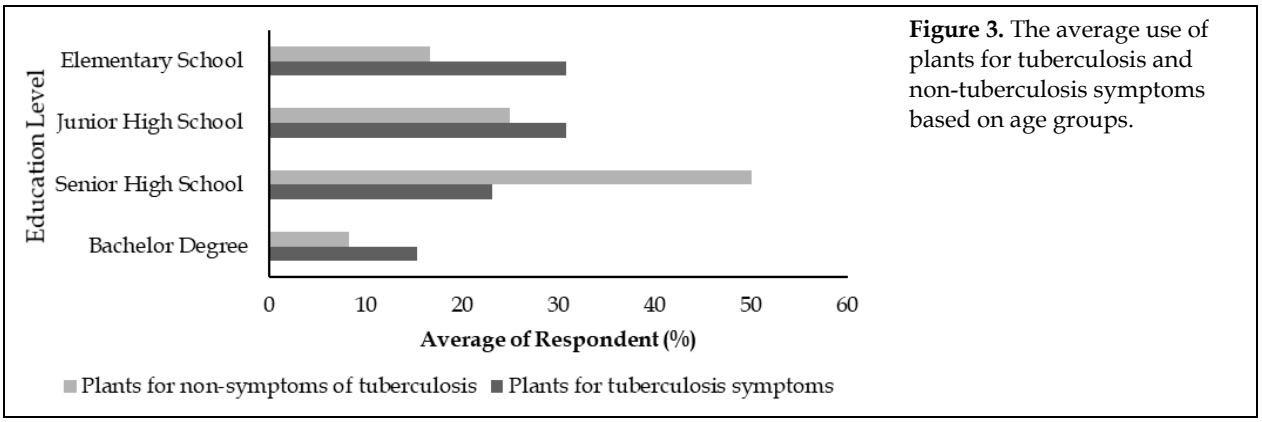

Figure 3. The average use of plants for tuberculosis and non-tuberculosis symptoms based on age groups.

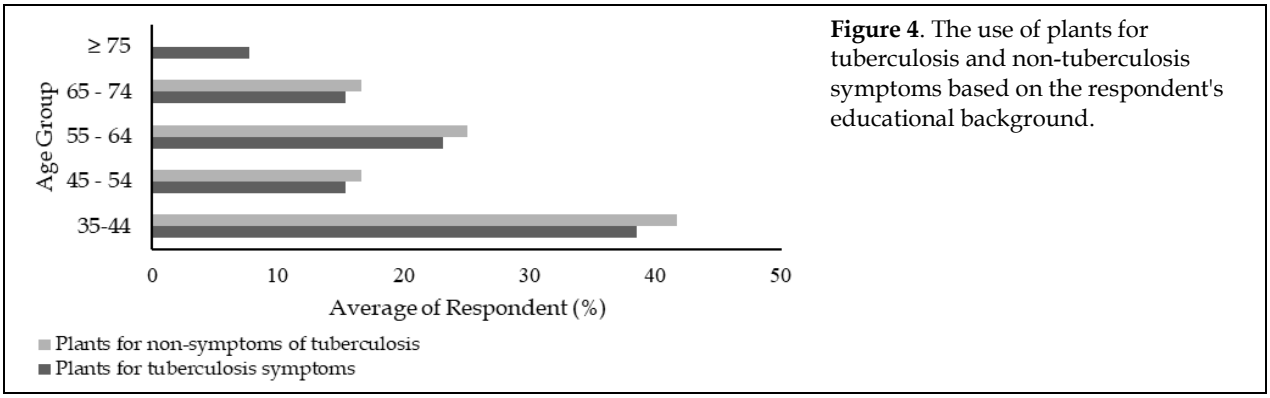

Figure 4. The use of plants for tuberculosis and non-tuberculosis symptoms based on the respondent's educational background.

The use of *Magnolia champaca* in Pamekasan District

Local people used *M. champaca* based on the type of illness or signs of tuberculosis. Based on the interviews with three respondents (key informants) who were the makers of herbal medicine, medicinal plants used by surrounding communities to treat diseases are shown in Table 1. Most people used medicinal plants for mothers' and children's health, especially *Piperaceae* and *Zingiberaceae* families. The *Piperaceae* family, specifically the *Piper refrofractum* Vahl., was one of the Pamekasan district community's primary commodities. In addition to mother and child health, some *Magnoliaceae* family plants were used by the community to treat coughs and fevers, which are tuberculosis symptoms. This plant was planted by the local community in the yard of the house. The plant part of the *Magnoliaceae* family that was often used by the community in making herbal medicine was the flower. The flowers contain several secondary metabolites such as flavonoids, triterpenoids, terpenoids and carotenoids, which have been described potentially as antituberculosis and anti-inflammatory agents (Sinha and Varma, 2016).

Identification of *Magnolia champaca* flavonoids as an anti-inflammatory candidate

Table 2 shown the compound of *M. champaca* based on a literature study and the approach to identify the compound. There were several identifications of flavonoids in the flower group. In line with Table 1., *M. champaca* flower parts have been widely used by local communities to treat diseases. The flavonoid groups have been widely identified (Table 2).

Flavonoid's identification of *Magnolia champaca* by molecular docking

The conformation of quercetin, rutin, kaempferol, (-)-epicatechins, and the enzyme COX-2 are shown in Fig. 5. It is indicated that there are native ligand NAG bonds (dark green) with a ligand copy of (-)-epicatechin and quercetin (dark green), but the EDO ligand is not bound by a ligand (Fig. 5).

Rutin, quercetin, kaempferol, and (-)-epicatechin form a conformation and spread over the A and B domains are shown in Fig. 6. There is a native ligand J8S (purple) bond with the enzyme p38 kinases, but a ligand copy of kaempferol does not bind with the native ligand, (-)-epicatechin, quercetin and rutin (green).

The conformation of rutin, kaempferol, (-)-epicatechin and quercetin (purple) with NF-κB protein are shown in Fig. 7. Ligand quercetin binds to kaempferol and (-)-epicatechin. The three ligands also form bonds with rutin ligand. Quercetin, kaempferol, (-)-epicatechin, and rutin when redocking simultaneously are fixed at the point as when the ligands are docking separately. Instead, the native OS6 ligand-bound four copies of the ligand. Native ligand OS6 (purple) stays in place as if the ligand were docking separately. While the EDO ligand (blue) is still in the role of redocking separately. Confirmation of ligand kaempferol, quercetin, (-)-epicatechin, and rutin (brown) with PI3K recognized that ligand copy and native ligand O4B (purple) remained in place as when redocking separately (Fig. 8).

The analysis was then obtained as Fig. 9 based on the STRING and STITCH pathways. Then these outcomes are translated to version 3.7.1 of Cyclope. Some proteins have direct involvement as an inflammatory mediator, especially those directly related to the activity of the quercetin, rutin, kaempferol, and (-)-epicatechin compounds. Many of these proteins such as CMC 2, contributes to COX production in the mitochondria. Then MAP3 K, which are a category of protein class kinases (derived from this class are p38 kinases and myd 88), PIK3KCA, which is a sub-unit of PI3K, and PNKD, which is involved in the activation of NF-κB, IL-4, TLR4, TNF, IL-13, IL-6 and IL-10.

Table 2. Flavonoid of *Magnolia champaca*.

Properties	Phytochemicals	Method	Ref.
Flower	Rutin and quercetin	HPLC	(Ananthi and Anuradha, 2015)
	Quercetin, kaempferol and (-)-epicatechin	GC-MS	(Malathi and Rajan, 2015)
Leaf	Quercetin	HPTLC	(Ahmad et al., 2011)
Stem bark	Quercetin	HPTLC	(Ahmad et al., 2011)

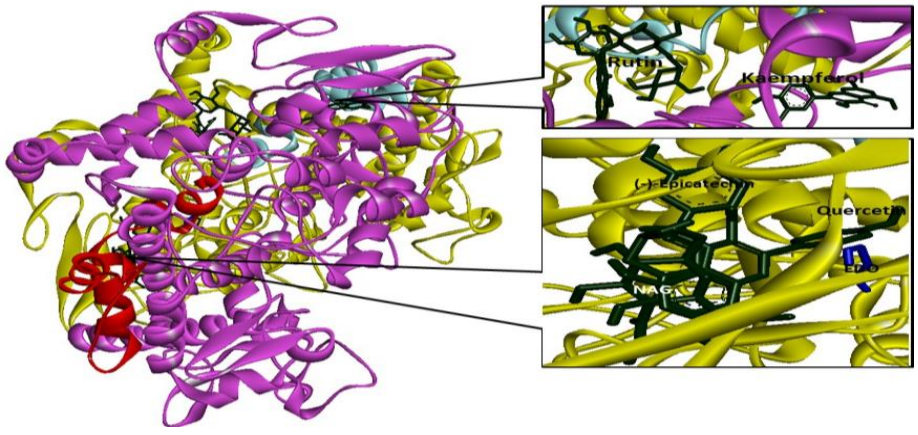

Figure 5. The complex of quercetin, kaempferol, rutin, (-)-epicatechin, and COX 2 protein in full position and zoom in.

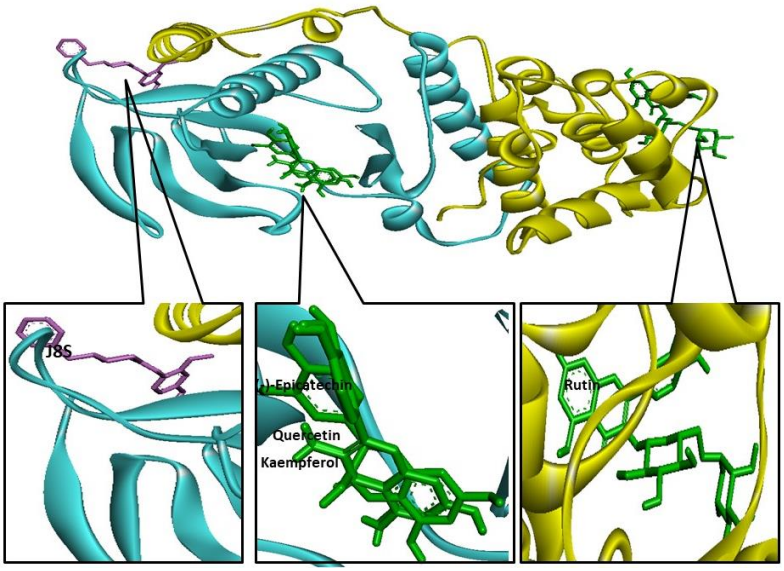

Figure 6. The complex of quercetin, kaempferol, rutin, (-)-epicatechin and p38 kinases in full position and zoom in.

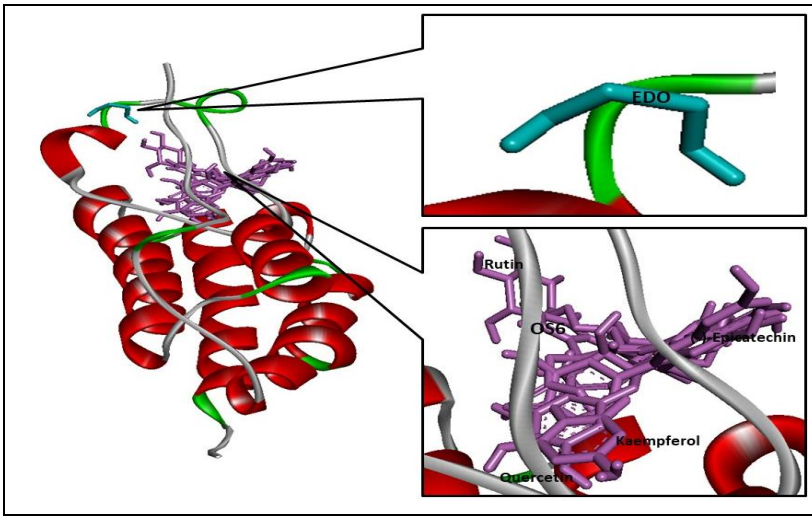

Figure 7. Complex ligand rutin, quercetin, kaempferol, and (-)-epicatechin with NF-κB protein in full position and zoom in.

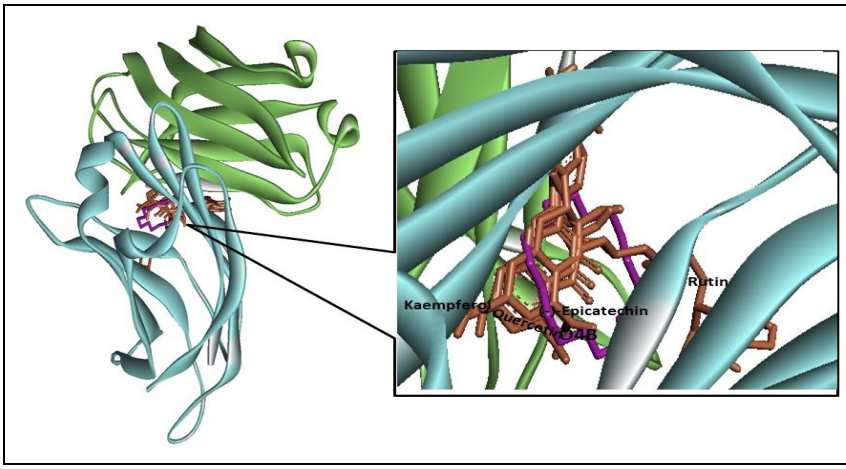

Figure 8. Complex ligand rutin, quercetin, kaempferol, and (-)-epicatechin with PI3K protein in full position and zoom in.

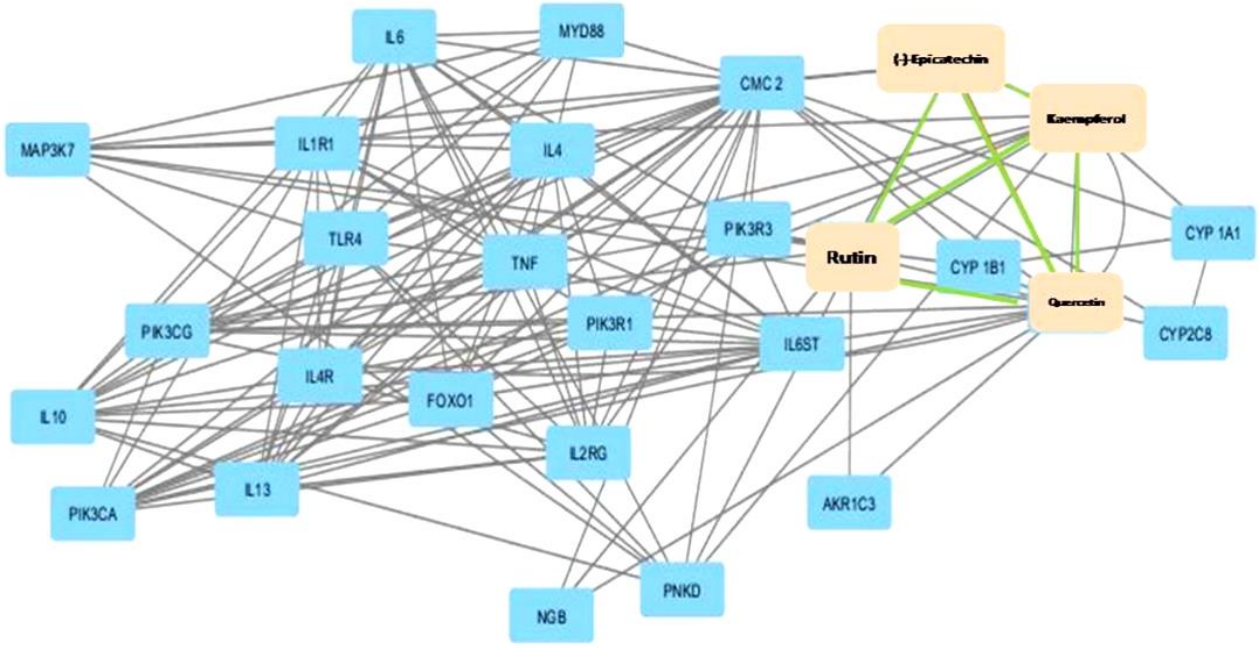

Figure 9. Networking active compounds with the inflammatory mediator.

In the local communities, *M. champaca* effectively prevents and treats fever, cough, and infection with traditional processing techniques (Sinha and Varma, 2016). Quercetin compounds were identified in the flower, leaf, and bark parts of *M. champaca*. Recently have been investigated six quercetin derivatives with anti-inflammatory activity, such as quercetin-3-O-γlucuronide, tamarixetin, isorhamnetin, isorhamnetin-3-O-γlucoside, quercetin-3,4′-di-O-γlucoside, and quercetin-3,5, 7, 3′, 4′-pentamethylether) (Lesjak et al., 2018). Quercetin derivatives are known to decrease inflammation due to reduced oxidative stress, modulating the expression of mRNA HO-1 and the release of TNF-α. Based on the activity, quercetin could be examined as an anti-inflammatory agent (Heeba et al., 2012).

Based on Fig. 5, ligand copy (-)-epicatechin switched from site B to site A when it was docking with quercetin and native ligand. Native ligand NAG has moved from site B to site A. Then, ligand copy rutin has moved from site A to site B due to its least capacity. The ligand copies of quercetin remained on site A. Native ligand NAG has moved from site B to site A. Kaempferol and quercetin remained in a position where separate docking and energy were both equal (Fig. 5). The EDO and NAG re-docking processes were carried out using the methods mentioned above. Comparison between NAG and EDO native ligand conformation based on X-ray crystal structure with re-docking results can be seen in Fig. 5. The binding energy of the copy ligand to the COX-2 proteins and the position of the residue of the amino acid can be seen in Table 3.

Table 3 shows the data binding energy ligand to protein, native ligand, and the amino acid residue position. COX-2 proteins have a native ligand NAG and EDO. Based on the data in Table 3, the low binding energy was found in quercetin (-9.1) and (-)-epicatechin (-9.0), which both compounds have a same amino acid residue in the interaction with COX-2. Quercetin interacts with COX-2 protein via several key amino acid residues, such as CYS 47 and GLN 461. These two amino acid residues also were found in the interaction of NAG and COX-2. It is indicated that quercetin could be considered as a selective COX-2 inhibitor (Miladiyah et al., 2017).

Table 3. Molecular docking of flavonoid compounds.

Protein	Domain	Ligand	Binding energy (Kcal/mol)	Amino acid residues
COX 2	A	Quercetin	-9.1	PRO 153, MET 48, VAL 46, GLY 135, CYS 36, PRO 40, HIS 39, **GLN 461**, LEU 152, GLY 45, **CYS 47**, TYR 130, ALA 151, ASP 125, ARG 469
	A	(-)-Epicatechin	-9.0	PRO 153, VAL 46, TYR 130, LYS 137, **CYS 47**, CYS 36, **GLN 461**, LEU 152, ARG 469, ARG 44, ASP 125, HIS 39, GLY 45
	B	Rutin	-9.9	PRO 542, ALA 543, LYS 369, LEU 366, PHE 367, TYR 373, GLN 370, **ARG 44**, ILE 124, ASP 125, HIS 122, SER 121, THR 118, PRO 127, SER 541, LYS 532, PHE 371, GLN 372
	B	Kaempferol	-9.1	TYR 130, ARG 44, VAL 46, PRO 153, **CYS 47**, CYS 36, GLY 135, TYR 136, LYS 137, ASP 125, ARG 469, GLY 45, **CYS 41**, LEU 152, **GLN 461**, HIS 39
		Native ligand		
	A	NAG	-6.9	GLU 465, **GLN 461**, **ARG 44**, **CYS 41**, **CYS 47**
	A	EDO	-3.3	TH2 212, GLN 454, TYR 148, HIS 386, THR 383, TYR 460, MET 458, ALA 379, ASN 382, PRO 218
P38 kinases	A2	Rutin	-7.5	**PRO 6**, ILE 346, TYR 342, ALA 93, THR 91
	A1	Kaempferol	-8.1	LEU 167, LYS 53, ALA 51, VAL 38, ILE 84, THR 106, TYR 35, GLU 71, ALA 157, LEU 156, ALA 111, SER 154, ASP 112, GLY 110, LEU 108, VAL 30, LEU 75, LEU 104
	A1	Quercetin	-7.7	ALA 157, ALA 51, VAL 38, TYR 35, LEU 167, LYS 53, GLU 71, ALA 111, THR 106, LEU 156, ILE 84, LEU 75, LEU 104, VAL 30, LEU 108, MET 109, GLY 110, ASP 112, VAL 158
	A1	(-)-Epicatechin	-7.7	THR 106, TYR 35, ALA 157, VAL 38, LYS 53, ALA 51, ALA 111, LEU 156, ASP 112, MET 109, ILE 84, VAL 52, LEU 104, LEU 75, LEU 86, LEU 167, VAL 30, GLY 110, GLY 31
		Native ligand		
	A1	J8S	-6.7	ARG 23, **PRO 6**, PRO 21, ARG 5, ARG 94
NF-κB	A	Rutin	-8.2	**LEU 92**, **PRO 82**, **VAL 87**, CYS 136, **ILE 146**, **PHE 83**, ASN 135, TYR 97, ASN 140, ASP 145, TYR 139, **TRP 81**, **LEU 94**, PRO 86, ASP 88, GLN 85
	A	Kaempferol	-8.3	**VAL 87**, **ILE 146**, **PRO 82**, **LEU 92, CYS 136**, ASN 135, TYR 139, TRP 81, **PHE 83**, MET 107, ASP 106, MET 105, TYR 97, MET 132, ASN 140
	A	Quercetin	-8.1	ASN 140, TYR 97, **ILE 146**, **VAL 87**, LEU 92, **PRO 82**, MET 107, ASP 106, MET105, PHE 83, MET `132, ASN 135
	A	(-)-Epicatechin	-7.6	TYR 97, **TRP 81**, **LEU 92**, **PRO 82**, **VAL 87**, **CYS 136**, MET 132, ASN 40, **ILE 146**, **LEU 94**, ILE 101, TYR 139, ASN 135, MET 105, **PHE 83**, GLN 65
		Native ligand		
	A	0s6	-7.6	**ILE 146**, **TRP 81**, **PRO 82**, **LEU92**, **PHE83**, **VAL 87**, **CYS 136**, **LEU 94**
	A	EDO	-3.0	TYR 98, LEU 94, LYS 99
PI3K	A	Rutin	-9.4	**GLU 1625**, **LYS 1613**, TYR 1595, **ILE 1614**, **LYS 1611**, **VAL 1628**, **MET 1626**, **LYS 1611**, THR 1612, LYS 1613, ARG 1610, ASN 1624, VAL 1628, **LEU 1627**, **LYS 1609**, THR 1612
	A	Quercetin	-8.2	**GLU 1625**, **VAL 1628**, **LYS 1611**, VAL 1627, ARG 1610, ASN 1624, **LYS 1613**, THR 1612, **MET 1626**
	A	Kaempferol	-8.0	**GLU 1625**, **VAL 1628**, ARG 1610, **LEU 1627**, **MET 1626**, **LYS 1611**, ASN 1624, THR 1612, **LYS 1611**, LYS 1613
	A	(-)-Epicatechin	-7.9	**GLU 1625**, **LEU 1627**, **VAL 1628**, **LYS 1611**, **MET 1626**, ARG 1610, **LYS 1613**, THR 1612
		Native ligand		
	A	O4B	-6.2	**GLU 1625**, **MET 1626**, **LYS 1609**, **ILE 1614**, ARG 1616, **LEU 1627**, **LYS 1613**, **LYS 1611**, LEU 1627, **VAL 1628**

Additionally, an amino acid residue MET 48 is present in quercetin and COX-2 protein interaction. The crystal structure of the COX-2 enzyme has a large region. Hydrophobic segments are filling with amino acid residues Met 48, Cys 47, Cys 36, which are thought to play a major role as the ligand entry point to the active site of the COX-2 enzyme, as shown in the crystal structure analysis of selective COX-2 inhibitors (Rakesh et al., 2016). The bond between the (-)-epicatechin ligand and the COX-2 protein is on the same residue of amino acids of native ligand and COX-2 proteins, such as GLU 465, ARG 44, and CYS 47. The result of redocking NF-κB also bears similarities with COX-2. This is due to the energy binding, and similarity of amino acids in native ligand OS6 resulting in ligand copy of quercetin, rutin, kaempferol, and (-)-epicatechin that bind the native ligand, but not all copy ligands may bind the native EDO ligand. These four ligand copies and native OS6 ligand are on TRP 146, ILE 81, LEU92, PHE83, VAL 87, CYS 136, LEU 94, and PRO 82 amino acid residues. The redocking PI3K analyses are known as quercetin's ligand copy, rutin, (-)-epicatechin, and kaempferol can bind native ligand O4B. It is suspected of having a binding energy factor and amino acid residue being identical. On the amino acid residue GLU 1625, LYS 1611, MET 1626, LYS 1609, and ILE 1614 are native ligand and ligand copies.

The bonds between p38 kinases enzymes with native ligand and quercetin, kaempferol, and (-)-epicatechin are on different amino acid residues. The result of redocking of Fig. 6 suggests no bond with all of the ligands copy between the native ligand. But ligand rutin has the similarity of amino acid residue with the native ligand, i.e., PRO 6. The rutin ligand location with the native ligand J8S is docked separately with the same site occupying proteins, which is A1. During the re-docking process, it is known that the rutin ligand is located on sites occupied by the native ligand J8S. When simultaneously docking, the ligand is known to have moved from site A1 to site A2. The location of the native ligand remains at site A1. Rutin ligand energy is smaller than J8S native ligand energy.

Quercetin, kaempferol and (-)-epicatechin have a same amino acids residue when interact with p38 kinases protein but these compounds showed a different binding affinity. When docking simultaneously as (-)-epicatechin, the quercetin ligand formed a bond (Fig. 6). It was assumed that rutin binding affinity on P38 kinases is smaller than the enzyme's native ligand J8S. Some factors that affect binding affinity, such as protein distance with ligand, protein or ligand flexibility, and protein and ligand hydrophobicity (Yunta, 2016).

Prostaglandin E_2 (PGE_2) is produced by COX-1 and COX-2. Both enzymes may cause inflammatory reactions. Therefore, those enzymes are the primary immunopathology mediator with the role of immune control on chronic infections. The macrophages and monocytes control COX-2 enzyme production in response to inflammatory signals. The Treg cell inhibits the T effector cell's function to suppress T cell immune response through a mechanism that relies on COX-2-PGE_2 (Tonby et al., 2016). COX is a responsible enzyme for transforming arachidonic acid (AA) into various prostanoids, including lipid mediators and extensive and diverse biological functions. COX exists in two major isoforms. The COX-1 is primarily a constituent and responsible for prostanoid formation. Then COX-2 is an isoform that contributes to prostanoids production in various growth and inflammatory processes. The eicosanoid synthesis begins after releasing AA via cytosol phospholipase A_2 from a phospholipid membrane. COX-1 and COX-2 are classified as G/H synthase 1/2, then converting AA to PG_2 and reducing it to PGH_2 (Chen and Smyth, 2011). Activation of p38 mitogen-activated protein kinases (MAPK) affects increased COX-2 expression. The two molecular events are related since MAPK 's pharmacological inhibition can decrease COX-2 expression (Parente et al., 2013).

MAPK activates in different cell surface receptors through double tyrosine phosphorylation and threonine, and involved in different cellular reactions. MAPKs and NF-κB play a crucial role in controlling the production of proinflammatory and anti-inflammatory cytokines in activated mac-

rophages. P38 is significant for the regulation of reduced IL-12p40 and IFN-γ. Increased levels of IL-12p40 in macrophages may inhibit ERK or p38 activity partly due to inhibition of IL-10 synthesis. Furthermore, the increased regulation of the IL-12P40 and the decline in the production regulation of IL-10 and NF-κB became significant (Zhang et al., 2005; Günzl et al., 2010). The PI3K is regulated by the cytokines IL-4 and IL-13. Then, PI3K activation will induce IL-10 cytokines. Anti-inflammatory cytokine response IL-10 can be modulated via PI3K-Akt-γSK3 line regulation (Antoniv and Ivashkiv, 2011).

CONCLUSIONS

The bioactive compounds of *M. champaca* such as quercetin, rutin, (-)-epicatechin and kaempferol might be considered as anti-inflammatory agents to treat inflammatory diseases, especially tuberculosis. Further *in vitro* and *in vivo* studies are needed to prove its therapeutic potential.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Maghfiroh K	Widyarti S	Batoro J	Sumitro SB
Concepts or ideas	x			
Design	x			x
Definition of intellectual content	x			
Literature search		x		
Experimental studies		x		
Data acquisition			x	
Data analysis			x	
Statistical analysis			x	
Manuscript preparation	x			x
Manuscript editing				x
Manuscript review	x	x	x	x

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