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Health benefits of aqueous and ethanolic extracts of medinilla speciosa blume

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ABSTRACT

Medinilla speciosa (local name: parijoto) has many pharmacological benefits, including for treating female infertility. This study aimed to identify bioactive compounds of the aqueous and ethanolic extracts of Medinilla speciosa; to find information on their pharmacological benefits, and to do its docking profile with the protein phosphatase1, which is associated with the enhancement of female fertility. Articles were searched from PubMed. The components of *Medinilla speciosa* were analyzed with LC-MS/MS. *In silico* study was conducted based PubChem, Protein Data Base, and Swiss ADME. Pyrx 0.8 and Discovery Studio Visualizer v21.1 were used to predict the interaction. Four flavonoids were identified, namely fisetin, robinetin, luteolin, and kaempferol. Except for robinetin, they exist in glycosidic form. One polyphenol, ellagic acid, was also identified. Literature studies showed they have various pharmacological benefits, such as antioxidants, anti-inflammation, anticancer, antidiabetes, organ protection, and antimicrobial. However, no information is available on its potential for fertility enhancement. Docking analysis showed that the bioactive compounds interact with the A and C chains of the catalytic domain of protein phosphatase I (PPI). Aqueous and ethanolic extracts of *Medinilla speciosa* possess fisetin, robinetin, luteolin, kaempferol, and ellagic acid that bind to catalytic chains of protein phosphatase I.

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INTRODUCTION

In Indonesia, mainly on Java Island and Bali, Medinilla speciosa (Melastomaceae) is an ornamental and medicinal plant. Traditionally, this plant is commended for various health care, such as fertility enhancement, diabetes, anticancer, diarrhea, mouth sores (sariawan), antiinflammation, anticancer, and antibacterial. Several species of Medinilla are traditionally used as various healthcare products. Its fruits and leaves are edible and good for female and male fertility enhancement (Wijayanti and Ardigurnita 2019). Ethnomedical uses of *Medinilla* related to several pharmacological benefits. *M. speciosa, M. magnifica,* and *M.myriantha* are known as parijoto in the valley of Colo village, Muria Mount, Kudus district, Central Java, Indonesia, as endemic plants. Parijoto is closely associated with the ethnic-majority area of Muria Mount. M. speciosa is a tropical plant used as traditional medicine by the community. It is well-known as an herbal medicine in this valley to overcome infertility.(Sugiarti and Pujiastuti 2017) It is an excellent nutritional supplement for pregnant women (Hanum, Prihastanti, and Jumari 2017). Generally, pregnant women consume parijoto after the gestational age enters five months and above. However, it can also be consumed from two to three months during pregnancy. The fruit and leaves have the potential to treat male fertility (Wijayanti and Ardigurnita 2019). Parijoto is also consumed to treat mouth sores (local term: sariawan) and antiinflamation (Sugiarti and Pujiastuti 2017), diarrhea, mouth sores, antiinflammation, anticancer, and anti-bacteria.

The aerial parts (fruits and leaves) of *M.speciosa* are supposed to have the potential to treat infertility. To predict this potential, in silico analysis can be applied. The detected bioactive compounds of M.speciosa extracts should be first identified. After identification, docking analysis can prove the characteristic of their interaction with protein phosphatase I (PP1) (Balakrishnan *et al.* 2019). This enzyme catalyzes dephosphorylation of eIF2α (eukaryotic translation initiation factor 2α). By inhibiting PP1, the phosphorylated eIF2α may continue its translation initiation. Balakrishnan, et al. (2019) reported that salubrinal, an eIF2α phosphatase inhibitor, can enhance eIF2α phosphorylation and improve fertility (Balakrishnan et al. 2019). Salubrinal can reduce ovarian immunoglobulin heavy chain binding protein (BiP) expression, rescue Pi3k/Akt signaling, and a doubling of primordial follicles. Salubrinal treatment can also normalize estrus cycle stage lengths. Salubrinal protects against primordial follicle loss, including suppressing the dephosphorylation of eIF2α, and that intervention significantly improves and extends ovarian function, fertility, and fecundity (Balakrishnan et al. 2019)(Choy et al. 2015).

Study on the bioactive compounds of *M.speciosa* is limited. Many bioactivity studies were conducted using plant extracts that were not followed with LC-MS/MS phytochemical analysis. This study aims first to identify major bioactive compounds of aqueous and ethanol extract of Medinilla speciosa with the help of LC-MS/MS. After identification, related articles were searched for their pharmacological benefits. Finally, molecular docking analysis was done to understand their interaction profiles with the protein phosphatase I, an enzyme that is associated with female fertility.

RESEARCH METHODS

Research Design

This research is an experimental study, using natural materials to explore the content of active compounds and their benefits. LCMS was used for analyzing the content of active compounds. Molecular docking was conducted using Pyrx 0.8 and discovery studio v21.1 The data analysis was conducted descriptively.

Population and Samples

Medinilla speciosa (local name parijoto) was taken from Muria Mount and grown in the garden of the university (Figure 1); the plant was identified by one of the authors according to the

determination keys of (Maxwell 1978). *M. speciosa* thrives and grows wild on mountain slopes or in forests on high humid and humid soils on mountain slopes starting at an altitude of 700 to 2,300 meters above sea level. One of the most common locations of the plant is found on the slopes of the Muria Mount, Colo Village, Dawe District, Kudus Regency, Central Java.

Figure 1. *Medinilla speciosa*

Instruments

The LC-MS/MS analysis was done using the Waters LCMS/MS-QTOF system. The Tof MSE operation mode was employed, featuring an Electrospray Ionization (ESI) source capable of both positive and negative ion modes. The column utilized for this purpose was a C18 column (specifically, the ACE HPLC Column with dimensions 25cm x 4.6mm).

Procedures

1. Plant extraction

Aerial parts of the plant were cut and air-dried. Then, 50 grams of the air-dried materials were boiled with 500 mL of hot distilled water at 90oC for 15 min (aqueous extract). After boiling, the water was separated from the solid material by filtering. The filtrate was then used for further analysis. Another 50 grams of the dried sample were macerated with 500 mL absolute ethanol for the ethanolic extract for 24 hours. The filtered macerate was evaporated with a Rotary Evaporator. Then, the extract was filtered. The filtrate was used for further analysis.

2. LC-MS/MS analysis

The LC-MS/MS analysis was done according to our previous method (Rahayu and Timotius 2022). LC-MS analysis was conducted using the Waters LCMS/MS-QTOF system. The mobile phases consisted of a solution containing 0.1% formic acid in acetonitrile and another with 0.1% formic acid in distilled water. These mobile phases were delivered at a 0.6 mL/min combined flow rate. To prepare the sample, 0.5 grams of the substance were dissolved in 10 mL of methanol and subjected to a 30-minute homogenization process in an ultrasonicator. The resulting suspension was then filtered through a 0.22 µm GHP/PTFE membrane filter. For injection into the LC-MS system, 10 µL of the filtered sample was utilized. The identification of active compounds within the samples via LCMS/MS-QTOF was carried out using the UNIFI software. This software is equipped with a mass spectrum library containing data on natural active substances from the Waters database. By comparing the mass spectrum of compounds within the sample to those in the library, UNIFI software determined the presence of specific compounds. The identification criteria included a mass error of analyte less than or equal to 5 ppm, isotope matches MZ RMS 6, analyte intensity of 300, and one fraction with a break value less than 4 in the fragment elucidation system.

3. Literature study

The searching step was carried out with the use of PubMed . The keywords were the names of the bioactive compounds detected by LC-MS/MS.

4. *In silico* analysis

We constructed models of the main predicted anti-targets PP1 using homologous modeling. Molecular docking studies were carried out with the obtained models. We performed molecular docking for all targets using AutoDock Vina, implemented in the PyRx 0.8 software package.

5. Ligands preparation

The bioactive compounds' structures was searched in the PubChem (https://pubchem.ncbi.nlm.nih.gov/compound). The compounds' basic structure (aglycone) was used in this docking. The chemical structures of Fisetin, Robinetin, Luteolin, Kaempferol, and Ellagic acid in 3D were acquired in sdf file format. Ligands were converted into the most stable structure energetically using energy minimization and then converted to pdbqt format using Open Babel in Pyrx

6. Protein preparation

The three-dimensional crystal structure of the PP1 enzyme (4XPN) was loaded in the PDB format from the protein molecules data bank (http://www.rcsb.org). Prior to docking, the molecules of all the non-proteinaceous components were removed. Hydrogen atoms were added throughout the protein structure before molecular docking using Discovery Studio v21.1.

7. Molecular docking procedure

The prepared protein was converted to pdbqt file in Pyrx 0.8. The positioning of the active site was determined with Discovery Studio to ascertain the X, Y, and Z coordinates, which were subsequently employed to generate grid boxes during the docking procedure facilitated by Pyrx. The dimensions of were set manually and amounted along the X-axis – 40Å, the Y-axis – 40 Å, and the Z-axis – 40 Å. These grid boxes effectively encompassed the entire active site of the protein structure, exploring potential protein-ligand interactions. All docking simulations were executed through Pyrx 0.8. Discovery Studio Visualizer v21.1 was employed to visualize the anchored complex structure fully (Chen et al. 2015; Le Donne et al. 2017).

8. Molsoft and Swiss ADME

Molsoft and Swiss ADME were used to find necessary data for Lipiski rule of five, drug-likeness, drug solubility, and binding affinity of the bioactive compounds.

Data Analysis

Data analysis is conducted descriptively with the aim of providing a clear overview of the data without delving into deeper statistical inferences. We collected data from LCMS, which consisted of active compounds, and then grouped these active compounds based on their active compound groups. The analysis results are succinctly summarized in a table. Similarly, with molecular docking results, the categorization of molecular interaction types is performed to conclude the data processing outcomes.

RESULTS

Five bioactive compounds were identified in the aqueous and ethanolic extracts of the aerial part of Medinilla speciosa. Four compounds (aglycon) were fisetin, robinetin, luteolin, and kaempferol except for robinetin, which was found in their glycosidic forms. In addition, a polyphenolic compound was found, namely ellagic acid (Figure 2, Table 1). Aqueous extract has a different composition compared with the ethanolic extract. Fisetin, robinetin, and luteolin exist in aqueous extract. Meanwhile, kaempferol and ellagic acid exist in ethanolic extract.

Information on the health benefits of the major bioactive compounds was obtained from various relevant published articles. Based on previous studies on the bioactive compounds from plant species, most of the compounds have antioxidant, antiinflammation, anticancer,

antimicrobial and antivirus, antidiabetic, and protective effects on cardiac, neuro, gastro, and liver. However no information is available on their ability to enhance fertility (Table 1).

Table 1. Major components in aqueous and ethanol extracts of Medinilla speciosa

Compound	A.E.	EtOH	Reported health benefits from other studies
Fisetin, 5,7,8,2′-Tetrahydroxy-flavone-7-O-β-D-glucoside	+	-	Antioxidant (Althunibat et al. 2019; Ma et al. 2019; Prasath, Sundaram, and Subramanian 2013) and antiinflammation (Althunibat et al. 2019; Ma et al. 2019) Anticancer: inhibits cell proliferation, induce apoptosis, and cell cycle arrest (Adan and Baran 2016), upregulates caspase-3(Ma et al. 2019) Antidiabetic (Althunibat et al. 2019); inhibit α-glucosidase(Jia et al. 2019; Shen et al. 2021); elevation in plasma insulin(Prasath, Sundaram, and Subramanian 2013) Cardioprotection (Ma et al. 2019) Antivirus(Zandi et al. 2011; Mishra, Kaur, and Singh 2022)
Luteolin-7-O-glucoside	+	-	Antioxidant (Sarikurkcu et al. 2020)and antiinflammation (Aziz, Kim, and Cho 2018) (Francisco et al. 2014) (Nabavi et al. 2015) Neuroprotection(Nabavi et al. 2015), protects dopaminergic neurons(Qin et al. 2019)
Robinetin	+	-	Antiinflammation(Germanò et al. 2015) Antihypertension(Asif et al. 2021) Antiangiogenic effects(Germanò et al. 2015) Antimicrobia (Cushnie and Lamb 2005)
Kaempferol glycosides: 7-O-α-L-Rhamnosyl-3-O-β-D-glucopyranosyl k aempferol Kaempferol 3-O-β-D-glucopyranoside 7-O-β-D-Glucopyrano-syl-kaempferol	-	+	Antioxidant (De Melo et al. 2009) (Calderón-Montaño et al. 2011) (Jung et al. 2009) and antiinflammation(De Melo et al. 2009) Neuroprotection(Calderón-Montaño et al. 2011), Antinociceptive (De Melo et al. 2009), anxiolytic, analgesic activities(Calderón-Montaño et al. 2011) Anticancer(Calderón-Montaño et al. 2011): induce apoptosis and inhibit breast cancer cells(Yi et al. 2016) inhibit proliferation, migration, and invasion of liver cancer(Yang et al. 2021) Antiarthritis(Aa et al. 2020) Antidiabetes(Calderón-Montaño et al. 2011): inhibit α-glucosidase(Amin et al. 2020) antiobesity(Zang et al. 2015) Antimicrobial, wound healing effect(Özay et al. 2019) Cardioprotection(Calderón-Montaño et al. 2011) (Hua et al. 2022), gastroprotection(Campos-Vidal et al. 2021) Antiosteoporotic, estrogenic/antiestrogenic, and antiallergic activities (Calderón-Montaño et al. 2011)
Ellagic acid	-	+	Antioxidant (Bharathi and Jagadeesan 2014) (Hwang et al.

		2010) and antiinflammation (Fu, Chen, and Guo 2020) (Corbett et al. 2010) (Ahad et al. 2014) Neuroprotection (Goudarzi et al. 2018), antidepressant (Dhingra and Chhillar 2012), antiepileptic (Dhingra and Jangra 2014), anxiolytic-like effect(Girish et al. 2013) Antidiabetic (Kyriakis et al. 2015): inhibit aldose reductase:(Akileshwari et al. 2014) Attenuates testicular disruption in rheumatoid arthritis (Arab et al. 2019) Antiplasmodium (Banzouzi et al. 2002), Antoporozoa (Belmares-Cerda et al. 2016), antiviral (Chen et al. 2015) (Le Donne et al. 2017) Antiangiogenesis effects (Chen et al. 2012) Cardioprotection (Elhemely et al. 2014), hepatoprotection(García-Niño and Zazueta 2015), osteoarthritis protection (Lin et al. 2020) Antiatherogenic (Mele et al. 2016)

Note: A.E.: aqueous

Table 2. Pharmacokinetic properties (ADME) of the identified bioactive compounds in Medinilla speciosa

Compound	TPSA	Water solubility (LogS)		Drug likeness Score	Binding activity (kcal/mol)	Molecular Weight (g/mol)	NHBa ≤ 10	NHBd≤ 5	Consensus LogP ≤ 5	MR 40-130	Lipinski's Rules Violation ≤ 1
		ESOL	ALI								
Fisetin: 5,7,8,2′-Tetrahydroxy-flavone-7-O-β-D-glucoside	111.13	Soluble	Soluble	0.46	-10.1	286.24	6	4	1.55	76.01	Yes. 0 violation
Luteolin: Luteolin-7-O-glucoside	111.13	Soluble	Moderate Soluble	0.38	-7.8	286.24	6	4	1.73	76.01	Yes. 0 violation
Robinetin	131.36	Soluble	Soluble	0.15	-7.9	302.24	7	5	1.12	78.03	Yes. 0 violation
Kaempferol: 7-O-α-L-Rhamnosyl-3-O-β-D-glucopyrano	111.13	Soluble	Soluble	0.5	-7.7	286.24	6	4	1.58	76.01	Yes. 0 violation

syl kaempferol Kaempferol 3-O-β-D-glucopyranoside 7-O-β-D-Glucopyranos-syl-kaempferol											
Ellagic acid	141.34	Soluble	Soluble	-1.11	-8.9	302.19	8	4	1.00	75.31	Yes. 0 violation

Table 3. Bound amino acids residue at A and C chain catalytic subunit of PPI

Bonding type	Ligands				
	Fisetin	Luteolin	Robinetin	Kaemferol	Ellagic acid
			Chain A		
Conventional hydrogen bond	none	SerA177 LeuA180 GlnA181 LysA234	AspA64 AspA92 AsnA124 HisA125 ArgA221 HisA248	AspA179	TyrA70 AsnA271
Van der Waals	none	LeuA176 ProA178 AspA179 ValA231 PheA235	HisA66 ArgA96 HisA173 TrpA206 GlnA249 PheA267 TyrA272	ArgA188 GlnA185 GlnA198 LeuA200	ProA24 GlyA67 GlnA68 TyrA69 GlyA97 GlnA99 ProA270 ProA298
Carbon hydrogen	none	TyrA216	None	none	none
Pi-PI Alkyl	none	None	PheA276		LysA98 AlaA299
			Chain C		
Conventional hydrogen	AspC64 ArgC96 ArgC221 HisC248	AspC92 ArgC221	None	GlyC135	none
Van der Waals	AsnC124 GlyC222 GlnC181 GlnC181 ValC223 LysC234 HisC66 HisC173 PheC267	AspC64 AsnC124 GlnC249 ValC223 ValC250 HisC66 HisC125 HisC173 PheC267	None	AspC138 ArgC96 ArgC142 GluC139 GlyC97 PheC136 TyrC134	none

	IleC130 TyrC134 TyrC272	TrpC206			
Carbon hydrogen	none	TyrC134 TyrC272	None	none	none
Pi-Pi Alkyl	TrpC206 HisC125	none	None	LysC98	none

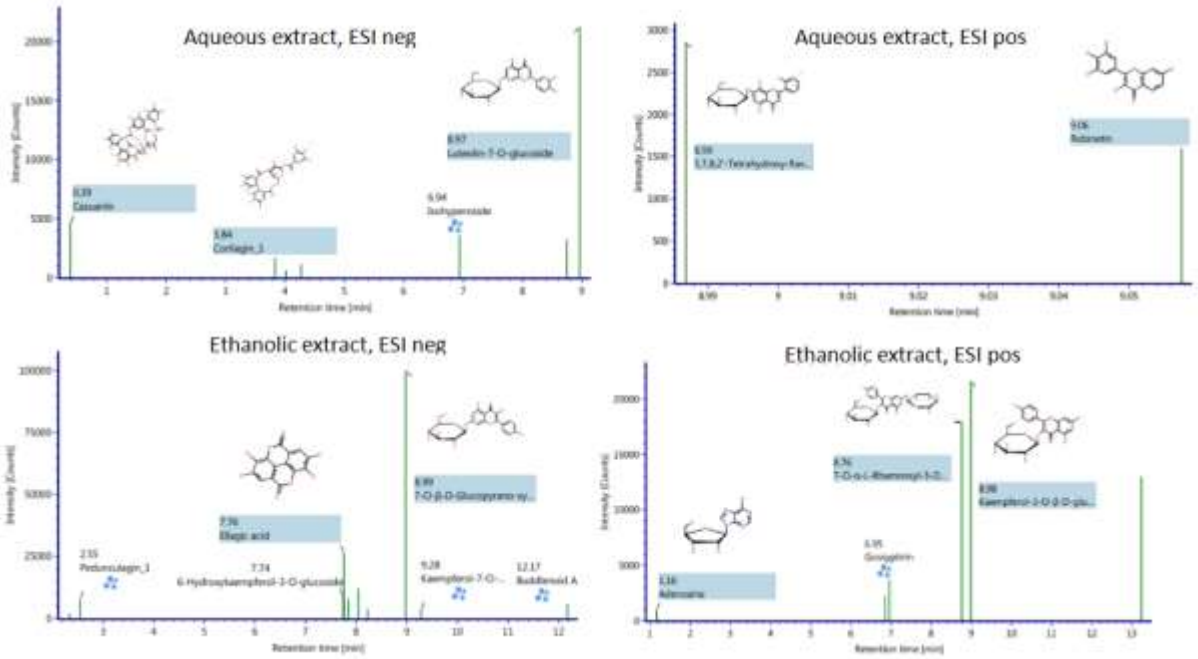

Figure 2. LC-MS/MS chromatograms of the aqueous and ethanolic extracts of *M.speciosa*

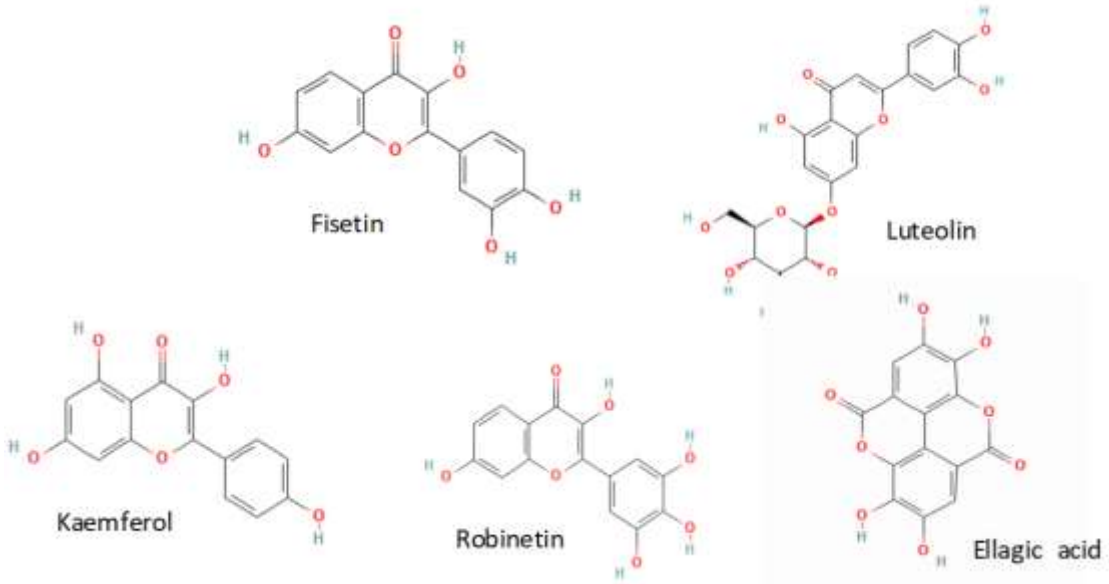

Figure 3. Chemical structure of the bioactive compounds from *M.speciosa*

DISCUSSION

Four flavonoids are major bioactive compounds of aqueous and ethanolic extracts from the aerial part of Medinilla speciosa. They are fisetin, robinetin, luteolin, kaempferol and ellagic acid, and for the fisrt time reported in this study (Figure 2, Figure 3 and Table 2). The last three compounds, luteolin and kaempferol (Diaz Sanchez 2017; Han *et al.* 2019), and ellagic acids (Hanh Nguyen *et al.* 2020) are found in Medinilla septentrionalis. However, the use of flavonoids medicinal agents is limited due to their poor stability, apparently limiting their bioavailability. The sugar moiety or glycosidic form has an important role in determining the absorption and bioavailability of flavonoids. Glycosylation of flavonoids is considered an effective method to rise their solubility and stability in water. Therefore, the presence of flavonoid glucosides in the extract is very beneficial (Hanh Nguyen *et al.* 2020).

The five bioactive compounds that exist in the extracts of *M. speciosa* have many pharmacological benefits (Table 1). Most pharmacological studies of the experimental activities have been carried out in vitro and/or in vivo with experimental animals. however, limited report is available on the clinical studies. The in vitro and in vivo experiments are covering beneficial for human health. such as antioxidative, anticarcinogenic, antiallergenic, antiinflammatory, antibacterial, antidiabetes, antimutagenic, and antitumor activities (Pertiwi *et al.* 2019; Tusanti, Johan, and Kisdjamiatun 2014; Sa'adah, Nurhayati, and Purwani 2018). From the best of our knowledge, there is no information available on the role of *M. speciosa* on the enhancement of fertility. Further research activities are needed to confirm the above mentioned benefits.

It is necessary to know whether bioactive compounds of *M.speciosa* have role in the enhancment female fertility and pregnancy health (Wijayanti and Ardigurnita 2019). In this study, inhibition of protein phosphatase I (PPI) was used as a model for this *in silico* study may help us to predict the strength of bioactive compounds. Table 2 shows that Fisetin has the highest binding affinity energy compared to other active compounds (-10.1 kcal/mol). The compound appears to have the potential to inhibit PPI activity. Table 3 shows that fisetin binds to amino acid residues in the domain C of PPI through Conventional hydrogen, Van der Waals, and Pi-Pi alkyl interactions. Ribonetin and ellagic acid bind to amino acid residues in domain A of PPI through Conventional hydrogen, van der waals and Pi-pi alkyl. Only luteolin is bound to the amino acid residue of the two domain (A and C).

ADME properties were assessed using swissADME to evaluate the molecules' solubility, bioavailability, and their potential to function as effective drugs. The analysis indicated that fisetin and three other compounds met the criteria of Lipinski rule without any violation and fell within an acceptable range concerning its bioavailability (Table 2). This compound has the ability to be absorbed and distributed within the body based on Lipinski's rule. The Lipinski rule sets specific limits for factors such as molecular weight (MW), the number of hydrogen bond acceptors and donors (HBA and HBD), and the water/octanol partition coefficient (log P). It was determined that compounds failing to meet two or more of these criteria are likely candidates for exclusion from further development. To achieve adequate drug absorption and distribution within the body, according to Lipinski's rule, the following criteria must be met: a maximum of 5 HBD, a molecular weight not exceeding 500 Da, a log P value no greater than 5, and a maximum of 10 HBA (Lipinski *et al,* 1997).

Unfortunately, using solubrinal, the known PPI inhibitor(Balakrishnan et al. 2019), control in docking process was not workable due to its three-dimentional (3D) conformer of salubrinal can not be generated. Salubrinal does not follow the criteria of 3D generation. Overall, this study shows that aqueous and ethanolic extracts from aerial part of *M.speciosa* can be predicted as suppressors of the de-phosphorylation of eIF2α, and that intervention in this way significantly improves and extends ovarian function, fertility, and fecundity.

CONCLUSION

The bioactive compounds of the aqueous and ethanolic extracts of *Medinilla speciosa* are flavonoids (fisetin, robinetin, luteolin, and kaempferol) and polyphenol (ellagic acid). These bioactive compounds have various pharmacological benefits, like antioxidant, antiinflamation, anticancer, antimicrobial, antidiabetes, and organs-protection. An *in silico* study predicted these bioactive compounds have the potential to inhibit protein phosphatase (PP1), an ovarium-key enzyme in fertility that is responsible for the dephosphorylation of eIF2α. These findings illustrate that aqueous and ethanolic extracts from *M. speciosa* are a promising therapeutic agent for the treatment of infertility. Therefore, the ethanol extract of *M.speciosa* is suitable anti infertility agent.

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