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ResearchArticle

# Gas Chromatography Mass Spectrometry Identification of Antiangiogenic Phytochemicals in *Aframomum danielli* K. Schum: An *In silico* Study

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**ABSTRACT**

*Aframomum danielli* is one of the African spices used in folklore medicine for the management of several diseases. This study identified the phytochemical components present in the n-hexane seed extract of the *A. danielli* by gas chromatography-mass spectrometry (GC-MS) analysis and also evaluated the antiangiogenic potential of the identified phytochemicals by performing molecular docking against human Vascular Endothelial Growth Factor (VEGF) and matrix metalloproteinases (MMP) using Molegro Virtual Docker. The GC-MS analysis identified the presence of phytochemical components β-Caryophyllene (RT: 18.479), α-Caryophyllene (RT: 19.189), (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone (RT: 22.976), N-Acetyl-m-aminobenzoic acid (RT: 31.651) and 3-Pyridineacetic acid (RT: 32.446). (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone were the strongest binding ligand (-65.744 kcal/mol for VEGF) and (-99.7836kcal/mol for MMP) while β-Caryophyllene was the weakest binding ligand. These compounds showed relative strong docking to VEGF with docking energies comparable to an anticancer drug, bevacizumab (-77.883kcal/mol for VEGF) and (-109.021kcal/mol for MMP). This *in silico* molecular docking study has shown that these phytochemical components could be responsible for antiangiogenic properties of *A. danielli*.

**Keywords**: Molecular docking, antiangiogenic, *Aframomum danielli*, cancer

## INTRODUCTION

Angiogenesis is a tightly controlled formation of new blood vessels from endothelium of pre-existing vasculature[1,2]. This process is physiologically involved in embryogenesis and wound healing. It is, however, also associated with the pathology of disease like cancer, retinopathies, rheumatoid arthritis as well as age related macular degeneration. Post-inflammatory factors such as hypoxia, reactive oxygen species, nitric oxide, IL-1β, IL-7, TNF-α have been reported to induce the expression of Vascular Endothelial Growth Factor (VEGF)[1,3]. VEGF is a direct angiogenic factor that stimulates cell proliferation and migration[4]. However, vascular endothelial cells require the degradation of surrounding tissue as part of the extracellular matrix remodeling. Among the extracellular matrix-degrading enzymes are matrix metalloproteinases (MMP)[5]. Hence, VEGF and MMP are critical proteins that influence tumor angiogenesis, local invasion and eventual metastasis. Inhibition of angiogenesis has been suggested as an attractive target for the treatment and control of cancer[1]. Great efforts are thus being made in the search for natural or synthetic agents capable of inhibiting carcinogenic processes[6-8]. A growing body of epidemiological and preclinical evidence points to culinary herbs and spices as minor dietary constituents with multiple anticancer characteristics[9,10]. Among approximately 180 spices commonly being used for culinary purposes globally, the anticancer properties of some, such as *Ocimum basilicum*, *Carum carvi*, *Amomum subulatum*, *Cinnamomum verum*, *Eugenia caryophyllata*, *Allium sativum* and *Rosmarinus officinalis* was reviewed by Kaefer and Milner[11]. They concluded that multiple processes including alterations of apoptosis and angiogenesis can account for the anticancer property of these spices. *Aframomum danielli* (Alligator pepper) is a natural North African spice commonly called *atare* in Yoruba Language. Although, the antioxidant, antimicrobial and enzyme-inhibitory properties of *A. danielli* has been reported[12-16], there is a dearth of information on its role in angiogenesis and cancer. This study therefore seek to identify the phytochemical components present in the n-hexane seed extract of the *A. danielli* by gas chromatography-mass spectrometry (GC-MS) analysis and also evaluated the anti-cancer potential of the identified phytochemicals by performing molecular docking against human VEGF and human MMP.

## MATERIALS AND METHOD

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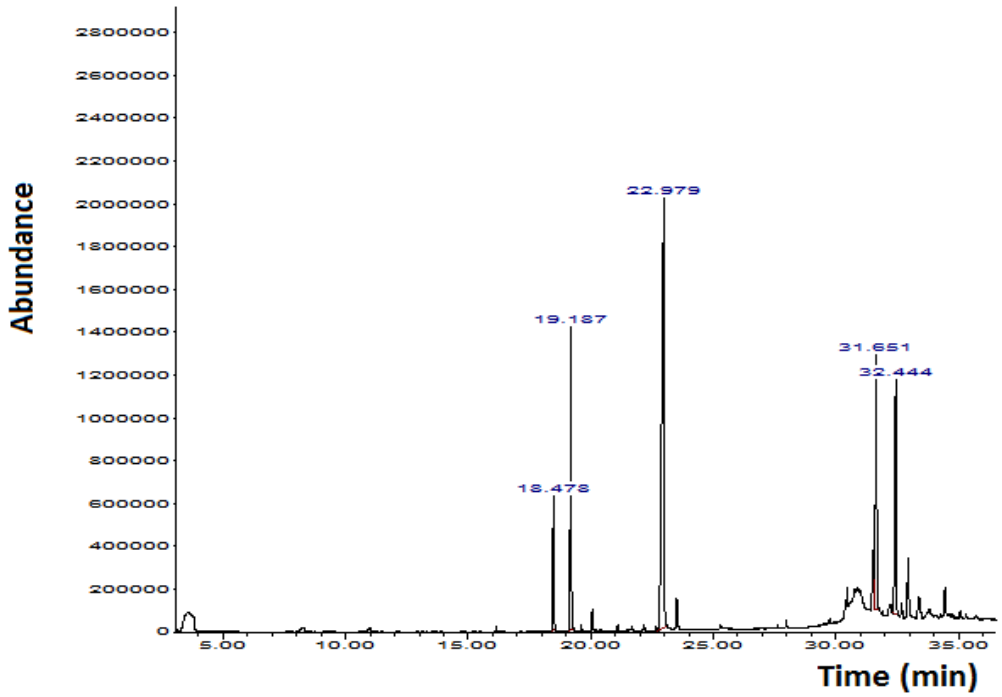



Figure 1.GC-MS chromatogram of n-hexane extract of *A. danielli*.

The spice was obtained from a local market. It was ground to fine powder and subjected to cold n-hexane extraction.

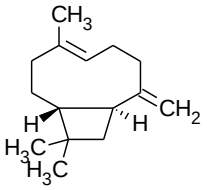
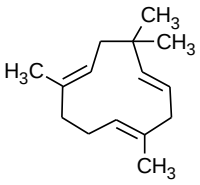
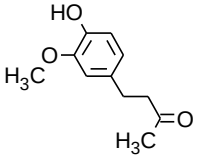
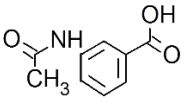
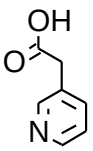
The GC-MS analysis was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC was equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1 ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 1 scan/s.

Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library.

The crystal structures (1.7 Å resolution) of the VEGF in complex with domain 2 of the Flt-1 receptor (PDB:1FLT) and the catalytic domain of human matrix metalloproteinase 10 (1Q3A), were obtained from the Protein Data Bank (PDB). Before Molecular Docking, the protein crystal structures were cleaned by removing the water molecules and hetero atoms.

Molecular docking of the ligands and Bevacizumab, an antiangiogenic drug, with MMP and VEGF was carried out using Molegro Virtual Docker (MVD) (17). MVD was first used to prepare the structure of the ligands and protein. It is based on a differential evolution algorithm; the

Table 1: Phytochemicals identified in n-hexane extract of *A. danielli*.

| PK # | RT | Compound | Structure |
|---|---|---|---|
| 1. | 18.479 | β-Caryophyllene |  |
| 2. | 19.189 | α-Caryophyllene |  |
| 3. | 22.976 | (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone |  |
| 4. | 31.651 | N-Acetyl-m-aminobenzoic acid |  |
| 5. | 32.446 | 3-Pyridineacetic acid |  |

solution of the algorithm takes into account the sum of the intermolecular interaction energy between the ligand and the protein, and the intramolecular interaction energy of the ligand. The docking energy scoring function ('dock score' refers to the approximate binding energies between protein and ligand, generally expressed in kcal/mol) is based on a modified piecewise linear potential with new hydrogen bonding and electrostatic terms included. Full description of the algorithm and its reliability compared to other common docking algorithm was described by Thomsen and Christensen [17].

Table 2: Molecular docking properties of the phytochemicals characterized from n-hexane extract of *A. danielli*.

| SN | Compound name | VEGF | | MMP | |
|---|---|---|---|---|---|
| | | MolDock Score | H-bond donor | MolDock Score | H-bond donor |
| 1 | β-Caryophyllene | -56.3114 | AsnV62(-0.338)<br>AspV63(-9.489)<br>GluV64(-12.499)<br>GlyV65(-2.085)<br>IleW46(-5.367)<br>Arg224Y(-10.178)<br>Gln225Y(-16.291)<br>His223Y(-2.389) | -84.0433 | Ala181 (-4.66885)<br>Asn240 (-0.596158)<br>Glu218 (-4.97248)<br>His178 (-1.2931)<br>His182 (-0.48002)<br>His217 (-14.4846)<br>Leu180 (-13.6473)<br>Leu213 (-1.66569)<br>Leu234 (-0.527533)<br>Leu238 (-6.55531)<br>Pro237 (-3.76143)<br>Ser179 (-5.62434)<br>Tyr236 (-0.994064)<br>Tyr239 (-8.69735)<br>Val214 (-9.65808)<br>Zinc (-1.89587) |
| 2 | α-Caryophyllene | -56.8469 | AspV63(-9.049)<br>GluV64(-8.386)<br>GlyV65(-2.126)<br>IleW46(-5.894)<br>Arg224Y(-8.410)<br>Gln225Y(-15.29)<br>His223Y(-2.869) | -84.2668 | Leu238 (-0.865011)<br>Ala181 (-6.58768)<br>Glu218 (-3.81815)<br>His178 (-2.88397)<br>His217 (-10.7865)<br>His221 (-0.444383)<br>His227 (-2.29188)<br>Leu180 (-13.8194)<br>Leu238 (-4.4712)<br>Pro237 (-6.3374)<br>Ser179 (-7.98394)<br>Tyr236 (-0.626717)<br>Tyr239 (-8.69562)<br>Val214 (-4.12492)<br>Zinc (-2.30682) |
| 3 | (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone | -65.7436 | Asp63V(-11.138)<br>Glu64V(-21.976)<br>Gly65V(-6.084)<br>Lys107V(-1.026)<br>Ile46W(-1.589)<br>Arg224Y(-14.978)<br>Gln225Y(-10.486)<br>His223Y(-1.376) | -99.7836 | Ala181 (-10.0272)<br>Asn240 (-6.02424)<br>Glu218 (-2.3851)<br>His178 (-0.57433)<br>His217 (-15.3847)<br>Leu180 (-16.0486)<br>Leu213 (-8.18876)<br>Leu234 (-2.82247)<br>Leu238 (-5.48889)<br>Pro237 (-4.08409)<br>Ser179 (-2.15499)<br>Tyr236 (-1.347)<br>Tyr239 (-17.9284)<br>Val214 (-10.2937)<br>Zinc (-1.05833) |

| | | | | | |
|---|---|---|---|---|---|
| 4 | N-Acetyl-m-aminobenzoic acid | -52.7117 | AspV63(-8.463)<br>GluV64(-6.163)<br>GlyV65(-0.759)<br>IleW46(-5.599)<br>Arg224Y(-9.138)<br>Gln225Y(-18.276)<br>His223Y(-5.914) | -77.8095 | Ala233 (-0.771163)<br>Asn240 (-6.38973)<br>Glu218 (-0.953132)<br>His217 (-15.4324)<br>Leu180 (-1.81538)<br>Leu213 (-9.44393)<br>Leu234 (-5.95709)<br>Leu238 (-9.16754)<br>Met235 (-0.330378)<br>Pro237 (-3.26195)<br>Tyr236 (-4.2783)<br>Tyr239 (-18.5873)<br>Val214 (-6.76835)<br>Zinc (-0.498486) |
| 5 | 3-Pyridineacetic acid | -56.4668 | Asp63V(-8.92271)<br>Glu64V(-10.4744)<br>Gly65V(-4.18456)<br>Arg224Y(-17.625)<br>Gln225Y(-12.7776)<br>His223Y(-0.69898) | -75.6223 | Ala233 (-1.5901)<br>Asn240 (-5.43409)<br>Glu218 (-0.774111)<br>His217 (-15.2462)<br>Leu180 (-2.35593)<br>Leu213 (-6.91661)<br>Leu234 (-7.74902)<br>Leu238 (-6.98033)<br>Met235 (-1.00615)<br>Pro237 (-3.18058)<br>Tyr236 (-4.84092)<br>Tyr239 (-18.37)<br>Val214 (-6.09268) |
| 6 | Bevacizumab | -77.8826 | AspV63(-9.88102)<br>GluV64(-11.2089)<br>GlyV65(-1.79158)<br>IleW46(-5.54677)<br>ProW85(-3.69805)<br>224Y(-16.8332)<br>225Y(-26.7513)<br>223Y(-1.66146) | -109.021 | Asn240 (-1.18898)<br>Leu238 (-2.30326)<br>Ser241 (-0.390526)<br>Tyr236 (-0.353609)<br>Tyr239 (-2.22988)<br>Ala181 (-3.73021)<br>Ala233 (-0.435821)<br>Asn210 (-0.32615)<br>Asn240 (-2.85984)<br>Glu218 (-1.80799)<br>His178 (-4.54551)<br>His217 (-17.3869)<br>Leu180 (-12.5349)<br>Leu213 (-6.99117)<br>Leu234 (-8.74449)<br>Leu238 (-8.58542)<br>Met235 (-0.363188)<br>Pro237 (-4.76675)<br>Ser179 (-10.2919)<br>Tyr236 (-3.60479)<br>Tyr239 (-18.8829)<br>Val214 (-8.74855)<br>Zinc (-0.983466) |

**RESULTS AND DISCUSSION**

The GC-MS analysis identified the presence of phytochemical components β-Caryophyllene (RT: 18.479), α-Caryophyllene (RT: 19.189), (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone (RT: 22.976), N-Acetyl-m-aminobenzoic acid (RT: 31.651) and 3-Pyridineacetic acid (RT: 32.446). A GC and GC-MS analysis of the leaves of some *Aframomum* spp. have reported the presence of essential oils rich in β-Caryophyllene and α-Caryophyllene[18]. Previous studies have identified these compounds as part of plant-derived anticancer essential oil component[19,20]. Gautam *et al*[20] reported the downregulation of matrix metalloproteases (MMP-6) and blockage of vascular endothelial growth factor receptor 1 by the essential oils of *Citrus sinensis* thereby making antimetastasis and antiangiogenesis as part of the mechanisms of anticancer action of the essential oil.

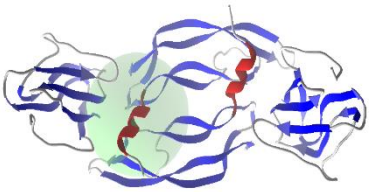


Figure 2a: Stereoview of the human VEGF (PDB: 1FLT). The binding pocket is highlighted in green.

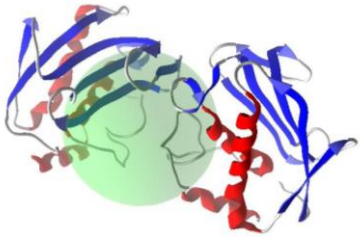


Figure 2b: Stereoview of the human MMP (PDB: 1Q3A). The binding pocket is highlighted in green.
MMP

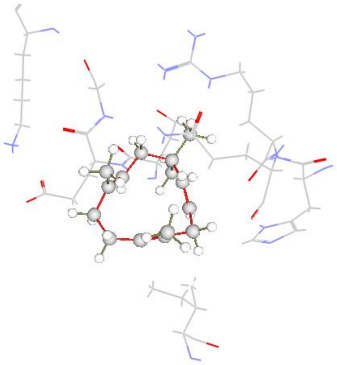


Figure 3a: Docking pose of β-caryophyllene to VEGF pocket

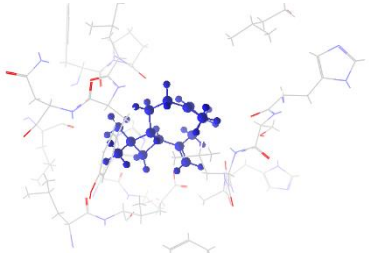


Figure 3b: Docking pose of β-caryophyllene to MMP pocket

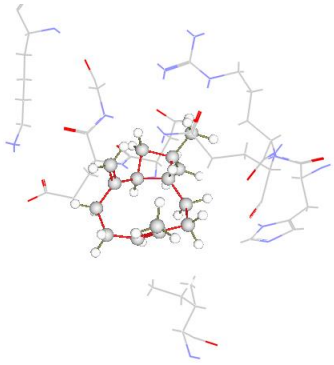


Figure 3c: Docking pose of α-caryophyllene to VEGF pocket

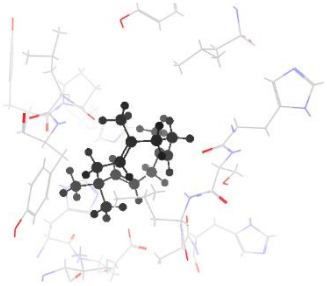


Figure 3d: Docking pose of α-caryophyllene to MMP pocket

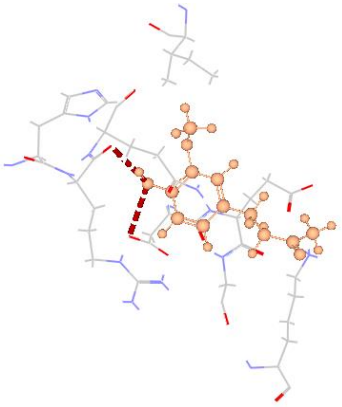


Figure 3e: Docking pose of (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone to VEGF pocket

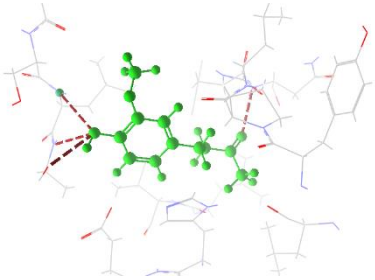


Figure 3f: Docking pose of (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone to MMP pocket

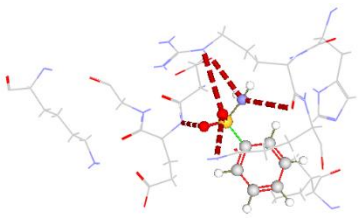


Figure 3g: Docking pose of N-Acetyl-m-aminobenzoic acid to VEGF pocket

These compounds showed relative stronger docking to MMP than VEGF. The docking energies were comparable to that of the antiangiogenic agent, bevacizumab that had binding energies of -109.021kcal/mol and -77.883kcal/mol to MMP and VEGF, respectively. 2-Butanone was the strongest binding ligand (-65.744kcal/mol for VEGF and -99.784kcal/mol for MMP) while β-caryophyllene was the weakest bind lingand (-56.311kcal/mol for VEGF and -84.043 for MMP). This implies that these compounds have the ability to bind to and directly inhibit the activity of MMP and VEGF. The role of MMP and VEGF as major mediators of angiogenesis in cancer has been reported[4,5]. The molecular docking results showed that all of these phytochemicals can directly bind to and inhibit the activity of MMP and VEGF (Figure 2). Figure 3 (a-l) shows the binding of the phytochemicals into the binding pocket of

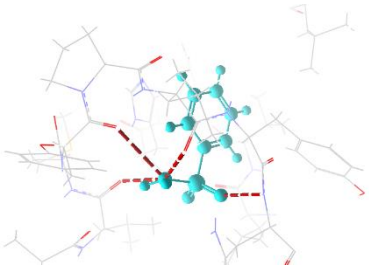


Figure 3h: Docking pose of N-Acetyl-m-aminobenzoic acid to MMP pocket

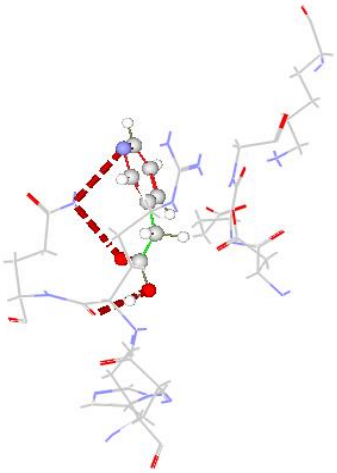


Figure 3i: Docking pose of 3-Pyridineacetic acid to VEGF pocket

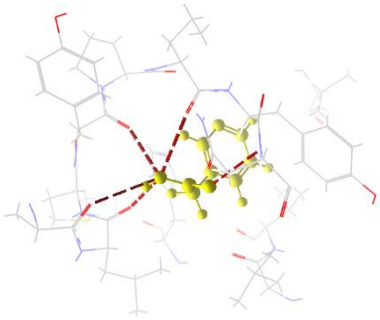


Figure 3j: Docking pose of 3-Pyridineacetic acid to MMP pocket

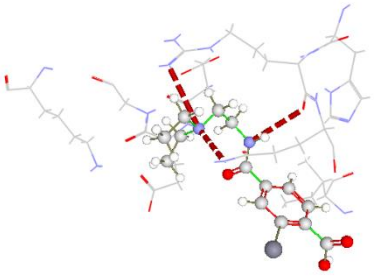


Figure 3k: Docking pose of Bevacizumab to VEGF pocket

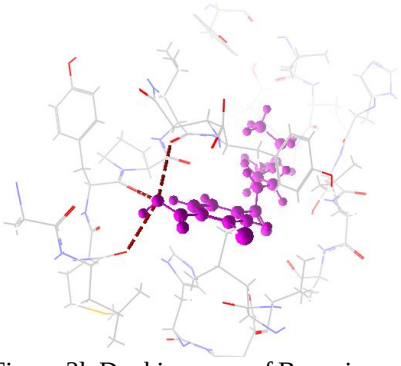


Figure 3l: Docking pose of Bevacizumab to MMP pocket

the human MMP and VEGF. The bindings are stabilized by hydrogens (H) bond(s) between some amino acid(s) present within the binding pockets of proteins and H-bond acceptors on the ligands. Generally, the interaction between ligands and MMP showed lowered MolDock score than that of VEGF. The MolDock scores and the atoms involved in the bond formation are shown in Table 2 with (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone having the best MolDock scores of -65.7436 and -99.784 for VEGF and MMP respectively. This is followed by 3-Pyridineacetic acid (-56.4668) for VEGF and N-Acetyl-m-aminobenzoic acid (-77.8095) for MMP. For both receptors, β-Caryophyllene had the highest binding MolDock of -56.3114 (for VEGF) and -84.0433 (for MMP). For MMP, the Zn atom (which is the cofactor for the enzyme) also participated in the bind of all the ligands except that of 3-Pyridineacetic acid. It is however noteworthy that none of the phytochemicals had a MolDock score as low as that of the antiangiogenic drug, Bevacizumab, which had the scores of -77.8826 and -109.021 for VEGF and MMP, respectively. Several studies have reported the anticancer and antiangiogenic properties of zingerone, a derivative of (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone[21,22]. Park *et al*[23] showed the anti-tumorigenic potential of caryophyllene and identified the down-regulation of VEGF as one of the mechanisms.

Several plant derived anti-angiogenic compounds known to suppress the expression and/or inhibit MMP and VEGF have been reported in literature. These plant species such as *Citrus sinensis*[24], *Schinus terebinthifolius*[25], *Curcuma zedoaria*[26], ginger[21] and *Psidium guajava*[23] contain several bioactive compounds like D-limonene, α-Pinene, Beta-elemene, zingerone and caryophyllene, respectively. Caryophyllenes and (4-Hydroxy-3-methoxyphenyl)ethyl methyl ketone, which is parent compound for zingerone, were also identified in n-hexane seed extract of the *A. danielli*. The use of *in silico* techniques has been shown to facilitate structure based drug design in drug development and discovery of mechanism of action of putative compounds even where high-throughput screening was unsuccessful[27] and the results obtained through molecular docking is predictive of biochemical activity[28]. This *in silico* molecular docking study has shown that these phytochemical components could be responsible for antiangiogenic property of *A. danielli*.

**CONFLICT OF INTEREST**

None declared

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