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ORIGINAL RESEARCH ARTICLE

Chemical constituents and their bioactivities of *Zingiber nimmonii*: An endemic species in South India

GREESHMA GOPALAN[1] • HRIDYA V KAVUNKAL[1] • DEVIKA S RAVINDRAN[1] • SABU MAMIYIL[3] • SHIBI IG[4]
RADHAKRISHNAN KV[1,2*]

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Abstract

*Eight major compounds were isolated and characterised from the rhizomes of Zingiber nimmonii (J. Graham) Dalzell by chromatographic and spectroscopic techniques. The compounds characterised by recording its Nuclear Magnetic Resonance Spectra (1D NMR, 2D NMR) together with Mass Spectra under ESI/HRMS and IR Spectra. The major compounds characterized as galangin-3-O-methyl ether (**1**), galangin-3,7-di-O-methyl ether (**2**), β-caryophyllene (**3**), α-humulene (**4**), zerumbone (**5**), germacrone (**6**), flavokavain B (**7**) and lupeol (**8**). Comparative DPPH scavenging, ABTS scavenging and NO scavenging assays of Zingiber nimmonii were carried out in both extracts and molecular level so as the consumption of this herb can regulate the free radicals produced in our body through various metabolic pathways. The compound, flavokavain B displayed better scavenging of DPPH radicals with IC_{50} values of 30.21 ± 0.111 µM, the compound, germacrone showed better scavenging of ABTS with an IC_{50} values of 51.7189 ± 0.688 µM, and the compound, zerumbone exhibited better IC_{50} value against the nitric oxide radicals with IC_{50} value of 97.876 ± 0.853 µM. The effects of inhibitory actions of the isolated compounds were also carried out for their virtual screening of antidiabetic and anticancer activities. The compounds **1** and **2** were effectively bound to the active sites of the enzymes with a docking score of -11.2018kcal/mol and -11.2703 kcal/mol respectively for the enzyme 2ITW and, -13.1593 kcal/mol and -11.9667 kcal/mol respectively for the enzyme 3A4A.*

*Corresponding author; Email: radhu2005@gmail.com
[1]Chemical Sciences and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram - 695019, India
[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad - 201002, India
[3]Department of Botany, University of Calicut, Calicut-35, India
[4]Department of Chemistry, Sree Narayana College, Chempazhanthy, Thiruvananthapuram-695587, India
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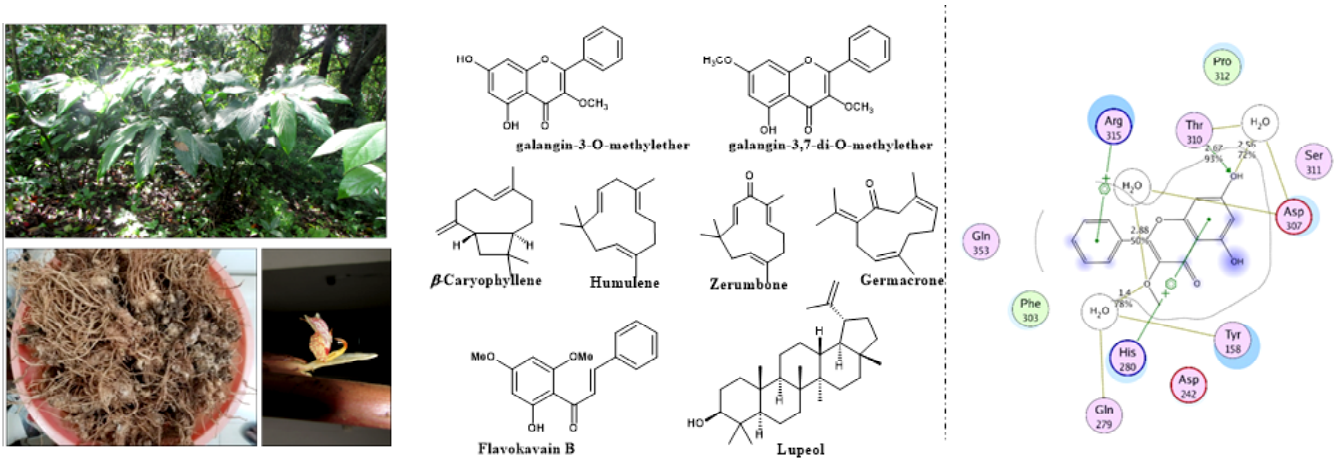
GRAPHICAL ABSTRACT



INTRODUCTION

The family *Zingiberaceae* is an important natural resource that constitutes a vital group of rhizomatous medicinal and aromatic plants, characterized by the presence of volatile oils and oleoresins of rich export value. India is one of the richest and diverse regions for *Zingiberaceae*, having 22 genera and about 170 species. The genus *Zingiber* has about 85 species of aromatic herbs, mostly distributed in East Asia and tropical Australia (Mabberley, 1990). It possesses various pharmacological and physiological effects and is an inevitable component in folklore medicines and serves as a raw material for various Ayurvedic formulations (Konickal, 2013). Classic examples for plants belonging to the genera include *Z. officinale* (ginger), *Z. zerumbet* (wild ginger), *Z. nimmonii, Z. cernuum*, etc.

Figure 1: *Zingiber nimmonii*-Flower, plant, and collected rhizomes

Zingiber nimmonii (J. Graham) Dalzell, is an endemic species of the Western Ghats in South India. It grows both at high and low altitudes and in moist areas under the shades of trees (Sabu, 2003). Its rhizomes are fleshy with a yellowish cross-section and an occasional purple tinge (Figure 1). The major constituents of the rhizome oil of *Z. nimmonii*, are isomeric sesquiterpenes, β-caryophyllene, and α-humulene (α-caryophyllene), varied from the rhizome oils of *Z. zerumbet* and *Z. officinale*, the most studied *Zingiber* oils (Sabulal et al., 2006). The extract level anti-microbial studies of the rhizome oil of *Z. nimmonii* showed significant activities against human pathogenic fungi, *Candida glabrata*, *Candida albicans,* and *Aspergillus niger*, (Sabulal et al., 2006). Finose and Gopalakrishnan (2014) studied the extract level antioxidant properties of this plant. Later, Govindarajan et al., (2016) had realised that the essential oil from *Z. nimmonii* can act as a newer and safer natural larvicide and repellent against malaria, dengue and filariasis mosquito vectors.

The overproduction of free radicals and their oxidative stress can cause chronic illness in the human body. The role of phytochemicals from medicinal plants are significant in this context due to its potentials associated with their antioxidant activity (Soobrattee et al., 2005; Zhang et al., 2015). Thus, this work focused on a detailed chemo-profiling of the rhizomes of *Z. nimmonii* and the phytochemicals isolated were first tested for its

antioxidative capacity by *in vitro* in both extract and molecular level, and then *in silico* screening of antidiabetic and anticancer activities were also done and described here.

MATERIALS AND METHODS

Characterisation of isolated compounds was carried out by different analytical techniques. The IR spectra were recorded with a Bruker FT-IR spectrometer. The nuclear magnetic resonance spectra (NMR) were recorded on a Bruker AMX 500 spectrophotometer ($CDCl_3$, CD_3COCD_3, MeOD and DMSO-d_6 as solvents). Chemical shifts for NMR spectra are reported as δ in units of parts per million (ppm) downfield from tetramethylsilane (δ 0.0) and relative to the signal of solvent. Mass spectra were recorded under ESI/HRMS at 60,000 resolutions using Thermo Scientific Exactive mass spectrometer and specific rotation was recorded using Jasco P-2000 polarimeter. Shimadzu UV-1800 spectrophotometer was used to measure the absorption maxima.

Collection of plant material

The fresh mature rhizomes of *Z. nimmonii* were collected from the Botanical Garden of University of Calicut, Malappuram District, Kerala, India during December 2017. The plant was authenticated by the Department of Botany, and a voucher specimen (voucher No. 103001) was submitted to the herbarium of Department of Botany, University of Calicut, Kerala, India.

Extraction and isolation

The dried fresh rhizomes of *Z. nimmonii* (~650g) was subjected to cold-extraction in hexane (3L for 3days; repeated twice) at room temperature followed by succeeding solvents acetone, ethanol, and water. The obtained extracts were concentrated in a Heidolph Rotary Evaporator at reduced pressure to get a crude of 1.4g for hexane, 16g for acetone, 2g for ethanol, and 1.8g for water. The acetone crude was subjected to Column Chromatography over silica gel (100-200mesh) eluting with increasing polarity of diverse gradient of solvents hexane-ethyl acetate (v/v) to get ten fraction pools which resulted in the isolation of eight compounds along with three common phytosterols and their glucosides. The structures of the isolated compounds were characterized by IR, 1D, and 2D NMR in combination with HRMS analysis together with the comparison of literature reports.

In vitro antioxidant studies

Evaluation of DPPH scavenging activity

The extract/compound/standard was added to methanolic solution of DPPH (2, 2′-diphenyl-picrylhydrazyl) and the reaction mixture was kept at 25°C for one hour in an incubator (Liu et al, 2007). The absorbance of the residual DPPH solution was determined at 517nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. Vitamin C was used as a positive control. The inhibition was calculated using the following formula, I (%) = 100 x $(A_0-A_1)/A_0$, where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard/compound, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value (Table 1).

Evaluation of ABTS scavenging activity

The ABTS (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation was prepared by mixing 2mM solution of ABTS and 70 mM potassium per sulphate in distilled water and used after 2 hrs for the assay (Re et al., 1999). To the various concentrations of extracts/standard/compound, 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer pH 7.4 were added and the absorbance was measured at 734 nm. Vitamin C was used as a positive control. The experiment was performed in triplicate. The inhibition was calculated in the following way, I (%) = 100 x $(A_0-A_1)/A_0$, where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value (Table 1).

Evaluation of NO scavenging activity

Nitric oxide (NO) scavenging activity was estimated by the Griess IlI osvoy reaction method (Garrat, 1964). The sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Greiss reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and its subsequent coupling with naphthyl ethylenediamine was read at 546nm, relative to the absorbance of standard solutions of potassium nitrite treated in the same way with Greiss reagent. The percentage nitrite radical scavenging activity of the extracts and gallic (standard) acid were calculated using the following formula, nitric oxide scavenged (%) = ($A_{control}$ – A_{test})/ $A_{control}$ × 100, where, $A_{control}$ = absorbance of the control sample and A_{test} = absorbance in the presence of the samples of extracts or standards (Table 1).

***In silico* antidiabetic and anticancer studies**

The purpose of this preliminary study was to analyse the effect of inhibitory action of the isolated compounds by computational docking studies, virtual screening of the compounds. The 3D structures of the proteins were downloaded from the Protein Data Bank (rcsb.org) and the molecular structures of the compounds were drawn using the software Chem Draw Professional 17.1 and were optimized using Molecular Operating Environment, MOE 2009.10. The molecular docking studies of all the compounds were carried out with a protein of PDB ID:3A4A (1.6Å resolution) with alpha-D-glucopyranose as their standard reference inhibitor for its antidiabetic action, and a protein of PDB ID:2ITW (2.88Å resolution) with 1,2,3,4-tetrahydrogen staurosporine (ITQ) as their standard reference inhibitor for its anticancer effects using MOE 2009.10. The default 'Site Finder' tool was used to identify the active site of the proteins. Using LigPlot analysis the binding interactions of the ligands to the proteins were studied and the docking results are given in Table 2 (Quing et al, 2014; Wermuth et al, 1998; Guner, 2002; Voet et al, 2014).

RESULTS AND DISCUSSION

Chemical constituents isolated

The eight major isolated compounds were structurally elucidated as follows:

Compound 1 was isolated as a yellow solid and is highly UV active. The IR spectrum (FT-IR (Neat) v_{max}: 3316, 1660, 1615, 1322, 1166 cm^{-1}) of the compound showed a broad and a sharp peak at 3316 and 1660 cm^{-1} corresponding to the hydroxyl and carbonyl groups respectively. The molecular formula, $C_{16}H_{12}O_5$ was deduced from (+)-HR-ESIMS analysis (m/z 285.0767 $[M + H]^+$, calcd: 285.0765) and ^{13}C NMR data. One proton singlet at δ 11.66 ppm in ^1H NMR indicates the presence of phenolic-OH with intramolecular H- bonding. The five aromatic protons, resonating in between δ 7.5-8.3 ppm, with interactions in HOMO COSY, can be attributed to a phenyl side chain. The two doublets at δ 6.38 and 6.50 ppm of coupling constant 2.0 Hz can be considered as a *meta*- coupled aromatic proton and they do not show any interactions with the methoxy carbon in HMBC. The peak at δ 175.4 ppm suggests the carbonyl group. Three proton singlets at δ 3.89 ppm indicate the presence of -OCH_3 group. DEPT-135 experiment revealed that the compound contains seven CH and one -CH_3 group. From all the above spectral details, and in comparison, with the literature reports, the compound 1 was confirmed as5,7-dihydroxy-3-methoxy-2-phenyl-4H-chromen-4-one or **galangin-3-*O*-methyl ether**(Jang et al, 2004).

Compound 2 was obtained as a yellow solid and is highly UV active. The IR spectrum of the compound showed peaks at 3268, 1647, 1611, 1162 cm^{-1}. The molecular formula, $C_{17}H_{15}O_5$ was deduced from (+)-HR-ESIMS analysis (m/z 299.0917) [M + H]+, calcd: 299.0916) and ^{13}C NMR data. The one proton singlet at δ 12.58 ppm in ^1H NMR indicates the presence of a phenolic -OH with intramolecular H- bonding. 6 proton singlets at δ 3.87 ppm indicate the presence of two -OCH_3 groups. DEPT experiment reveals that the compound contains six CH and two -CH_3 groups. Also, in the carbon NMR, the peak at δ 178.9 ppm indicates the presence of a carbonyl group. While

comparing the spectral features of compound **2** with that of compound **1**, it was found that they differ only in the presence of an excess methoxy group. Hence, based on its spectral data and literature reports, the structure of compound **2** was confirmed as 5-hydroxy-3, 7-dimethoxy-2-phenyl-4H-chromen-4-one or **galangin-3,7-di-O-methyl ether** (Jang et. al., 2004).

Compound 3 was obtained as a viscous oil, with a pleasant smell. The IR spectrum of the compound showed broad and sharp peaks at 3066, 2917, 1124 cm^{-1}. The molecular formula, C15H24 was deduced from (+)-HR-ESIMS analysis (m/z 205.1960 [M + H] +, calcd: 205.1956) and ^{13}C NMR data. In the proton NMR, the molecule showed three methyl groups, along with twelve more aliphatic protons. The spectrum seemed to be an isomeric mixture, due to the repetition of almost all the peaks in less than 10 % of the major compound (64 mg). In the carbon NMR, the molecule showed a total of fifteen carbons, of which six are $-CH_2-$ in nature. Also, the detailed studies of various NMR spectra showed that the molecule possesses one exocyclic double bond and is bicyclic. From the detailed analysis, the compound was confirmed as ***β*-caryophyllene**, a natural bicyclic sesquiterpene that is a constituent of many essential oils. The isomeric mixture may probably be isocaryophyllene (the *cis* double bond isomer), which is usually found as a mixture with β-caryophyllene. Caryophyllene is notable for having a cyclobutane ring, as well as a *trans*-double bond in a 9-membered ring, both rarities in nature (Fidyt et al, 2016).

Compound 4 was isolated as a colourless viscous liquid with intense UV activity (22 mg). The IR spectrum (**FT-IR** (Neat) ν_{max}: 2711, 1662, 1443, 1354, 1178, 969, 824 cm^{-1}) of the compound showed signals in between 1660-1700 cm^{-1}, corresponding to the C=C stretching frequency. The molecular formula, $C_{15}H_{24}$ was deduced from (+)-HR-ESIMS analysis (m/z 205.1959 [M + H] +, calcd: 205.1956) and ^{13}C NMR data. In the proton NMR spectrum, the signals due to the olefinic protons were noticeable as a multiplet in between δ 5.62-5.56 ppm and as a doublet resonating at δ 5.15 ppm with a coupling constant of 16.0Hz. The two triplets at δ 4.95 and 4.87 ppm are also representing olefinic protons. The singlets at δ 1.64, 1.43, and 1.06 ppm were attributed to four methyl groups. In the ^{13}CNMR spectrum, the carbons that resonated at 141.1, 139.3, 133.2, 127.7, 125.8, and 125.0 ppm correspond to olefinic carbons, and among them, four are protonated. The presence of four CH, four $-CH_{2-}$ and four $-CH_3$ were confirmed using the DEPT-135. From various spectral analyses, the structure of the compound was confirmed as α-caryophyllene or **humulene**, and the spectral data were in good agreement with the literature reports (Fidyt et al., 2016).

Compound 5 was isolated as a colorless crystalline solid with intense UV activity. The IR spectrum (**FT-IR** (Neat) ν_{max}: 3026, 2964, 2924, 2856, 1656 (dienone), 1455 cm^{-1}) showed a strong absorption at 1656 cm^{-1} indicating the presence of a dienone/amide moiety (18 mg). The molecular formula, $C_{15}H_{22}O$ was deduced from (+)-HR-ESIMS analysis (m/z 219.1748 [M + H] +, calcd: 219.1749) and ^{13}C NMR data. The ^{1}H NMR spectrum showed the presence of four olefinic protons as two doublets at δ 5.96 and 5.86 ppm with a coupling constant of 16.0Hz and two multiplets between d 5.50- 6.50 ppm. Four singlets at δ 1.08, 1.21, 1.54, and 1.79 ppm, each accounting for three protons could be readily identified as four methyl groups. With the presence of fifteen carbons in the ^{13}C spectrum, along with the resemblances with previous compounds, the molecule was confirmed as a sesquiterpene. DEPT-135 experiment revealed that the compound contains three $-CH_2-$ and four $-CH_3$ groups. The key feature of the spectrum was the signal at δ 204.0 ppm indicating the carbonyl group. Also, the mass spectrum showed a molecular ion peak at m/z 241.1567 corresponding to $[M+Na]^+$. From the detailed spectral analysis and on comparison with literature reports, the compound was identified as **zerumbone/**(2E,6E,10E)-2,6,9,9-tetramethyl-cycloundeca-2, 6,10-trienone (Dev et al, 1960; Damodaran et al, 1965 & 1968).

Compound 6 was isolated as a colourless solid. The strong absorption at 1688 cm^{-1} in the IR

spectrum (**FT-IR**(Neat) v_{max}: 2962, 2933, 1688 (enone), 1466 cm^{-1}), indicates the presence of an α, β-unsaturated carbonyl moiety. The molecular formula, $C_{15}H_{22}O$ was deduced from (+)-HR-ESIMS analysis (m/z 219.1740 $[M + H]^+$, calcd: 219.1749) and ^{13}C NMR data. In the proton NMR, the molecule showed two multiplets in between δ 4.50-500 ppm, which indicate the presence of two olefinic protons. Also, comparing with the carbon and other 2D NMR techniques, it was found that the molecule possesses four methyl (at δ 1.78, 1.73, 1.63 and 1.44 ppm) and four -CH_2- groups (carbon at δ 55.9, 38.11, 29.2 and 16.7 ppm), which are aliphatic in nature. The peak at δ 207.9 ppm in the carbon NMR indicates the presence of a carbonyl group as in the case of zerumbone. The presence of three double bonds were also confirmed from the four quaternary carbons in the olefinic region (by comparing the DEPT-135 NMR with ^{13}C NMR). A total of fifteen carbons in the carbon NMR is suggestive for a sesquiterpenoid moiety. From detailed spectral analysis, and comparison with previous literature reports, the compound **6** was identified as **germacrone/** (3Z,7Z)-3,7-dimethyl-10-(propan-2-ylidene) cyclodeca-3,7-dienone (4 mg) (Ahmed Hamdi et al, 2015).

Compound 7 was isolated as a pale yellow coloured solid (14 mg). The molecular formula, $C_{17}H_{16}O_4$ was deduced from (+)-HR-ESIMS analysis (m/z 285.1086 $[M + H]^+$, calcd: 285.1107) and ^{13}C NMR data. In the IR spectra (**FT-IR**(Neat) v_{max}: 3341, 2962, 2997, 2935, 1691(enone), 1466 cm^{-1}), the molecule showed an intense absorption at 1691 cm^{-1}, which indicates the presence of an α, β-unsaturated carbonyl or an amide moiety. The proton, as well as carbon NMR of the molecule, showed much resemblance with the spectral data of compounds **1** and **2**. Unlike compound **2**, this molecule carried two distinct methoxy groups at δ 3.93 and 3.85 ppm. The two doublets at δ 7.91 and 7.79 ppm with a coupling constant of 15.5 Hz, clearly indicate the presence of a *trans* olefinic proton. Also, the singlet proton at δ 14.29 ppm is clearly attributed to a hydroxyl group, with hydrogen bonding interactions. In the carbon NMR, the peak at δ 192.7

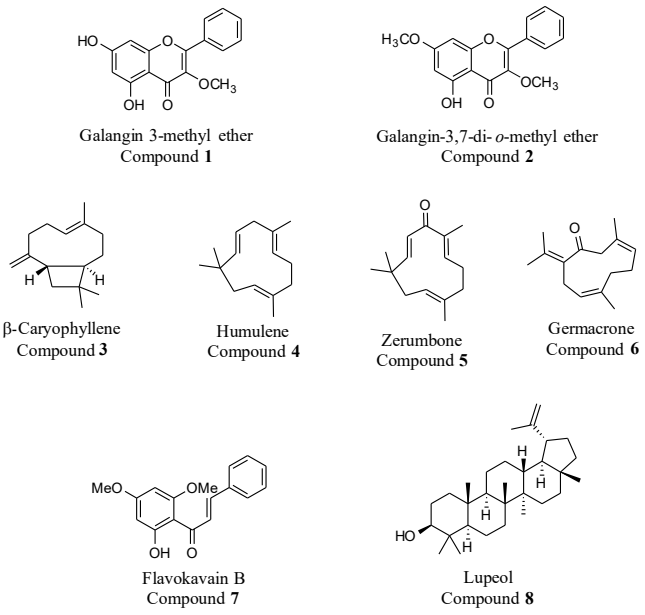


Figure 2: Isolated compounds of *Z. nimmonii*

ppm supports the presence of a flavone carbonyl group. Finally, by the comparison of spectral data of the compound with that of literature reports, the structure of the molecule was confirmed as **flavokavain B** [(*E*)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one] (Rodrigues et al., 2017).

Compound 8 was isolated from the fraction pools 5-8, as white crystalline needles with a melting point of 119-121°C (14 mg). In the IR spectrum, the intense absorptions at 3449, 2931, 2311, 1587, 1258 and 899 cm^{-1} displayed by the compound clearly indicated that it consists of hydroxyl and olefin functionalities. The molecular formula, $C_{30}H_{50}O$ was deduced from (+)-HR-ESIMS analysis (m/z 427.3943 $[M + H]^+$, calcd : 427.3940) and ^{13}C NMR data. The presence of an exocyclic olefinic system in the molecules was confirmed from two singlets at δ 4.70 and 4.57 ppm with carbon at δ 109.3 ppm. The ^1H NMR spectrum also revealed the presence of seven tertiary methyl protons in between δ 0.77 and 1.65 ppm. Finally, the structure of the compound **8** was accomplished as the plant sterol, **lupeol,** through various 2D NMR experiments (COSY, HMQC and HMBC) (Abdullahi et. al., 2013).

Table 1: IC_{50} values of different extracts towards various radical generators

Extracts	IC_{50} values (μg/mL)		
	DPPH radical scavenging	ABTS radical scavenging	NO radical scavenging
Hexane	1.987 ± 0.201	60.6493 ± 0.76	90.010 ± 0.332
Acetone	9.0043 ± 0.546	117.386 ± 0.943	91 ± 0.831
Ethanol	2.01 ± 0.112	110.386 ± 0.221	119 ± 0.563
Water	3.12 ± 0.887	117.413 ± 0.269	110 ± 0.731
Ascorbic acid	6.878 ± 0.231	14.1254 ± 0.461	-
Gallic acid	-	-	55.84 ± 0.876

In vitro Antioxidant Studies

Extract level *in vitro* Antioxidant Studies

Phytoconstituents with natural redox properties were determined in *Z. nimmonii* extract by DPPH, ABTS and NO radical scavenging assays (Carlsen et al, 2010). Out of the three assays, DPPH radical scavenging is effectively good in comparison with others. DPPH is a stabilized radical, while ABTS radical has to be formed initially. The hexane extract of *Z. nimmonii* exhibited the lowest IC_{50} against all the three radicals (IC_{50} = 1.987 ± 0.201 µg/mL for DPPH, 60.6493 ± 0.76 µg/mL for ABTS and 90.010 ± 0.332 µg/mL for NO radicals), followed by acetone, ethanol and water extracts (Table 1). The extracts demonstrated significant scavenging of DPPH radicals, their activity against ABTS and NO radicals were comparatively poor. This might be due to the difference in solubility towards the medium of the active constituents present in each extract. The number and molecular size of the components in each extract should have taken into consideration.

Molecular level *in vitro* antioxidant studies

The molecular level studies found that most of these isolated compounds effectively scavenge the DPPH and NO radicals with comparatively better IC_{50} values than the standard compounds, ascorbic acid and gallic acid. Among the compounds studied, compound **2** and flavokavain B displayed the better scavenging of DPPH radicals with IC_{50} values of 33.76 ± 0.651 μM and 30.21 ± 0.111μM, respectively. Amongst the flavonoids, chalcones have been identified as interesting compounds possessing higher antioxidative potentials (Won et al., 2005), and the presence of a flavone carbonyl group and a hydroxyl group supports this value. Coming to the ABTS assay, germacrone and compound **1** demonstrated the better IC_{50} values (51.7189 ± 0.688 μM and 53.506 ± 0.811 μM respectively) followed by zerumbone (57.8181 ± 0.751 μM) and compound **2** (69.0536 ±

Table 3: Molecular docking studies

Compounds	E-score kcal/mol	
	2ITW	3A4A
Galangin 1	-11.2018	-13.1593
Galangin 2	-11.2703	-11.9667
Caryophyllene	-6.36811	-8.03946
Humulene	-6.5089	-9.08878
Zerumbone	-5.97459	-8.35367
Germacrone	-6.75311	-9.23364
Flavokavain B	-9.84695	-11.4503
Lupeol	-9.08736	-10.7358

Table 2: *In vitro* antioxidant potential of isolated compounds

Compounds	IC_{50} values (μM)		
	DPPH radical scavenging	ABTS radical scavenging	NO radical scavenging
Galangin 1	35.08 ± 0.54	53.506 ± 0.811	114.29 ± 0.12
Galangin 2	33.76 ± 0.651	69.0536 ± 0.713	111.8743 ± 0.87
Caryophyllene	46.77 ± 0.813	189.0842 ± 0.251	226.87 ± 0.93
Humulene	44.87 ± 0.366	250.2597 ± 0.912	262.81 ± 0.331
Zerumbone	40.08 ± 0.951	57.8181 ± 0.751	97.876 ± 0.853
Germacrone	41.88 ± 0.776	51.7189 ± 0.688	213.05 ± 0.571
Flavokavain B	30.21 ± 0.111	77.8321 ± 0.03	189.01 ± 0.616
Ascorbic acid	39.01 ± 0.12	80.17 ± 0.61	-
Gallic acid	-	-	320.0 ± 0.27

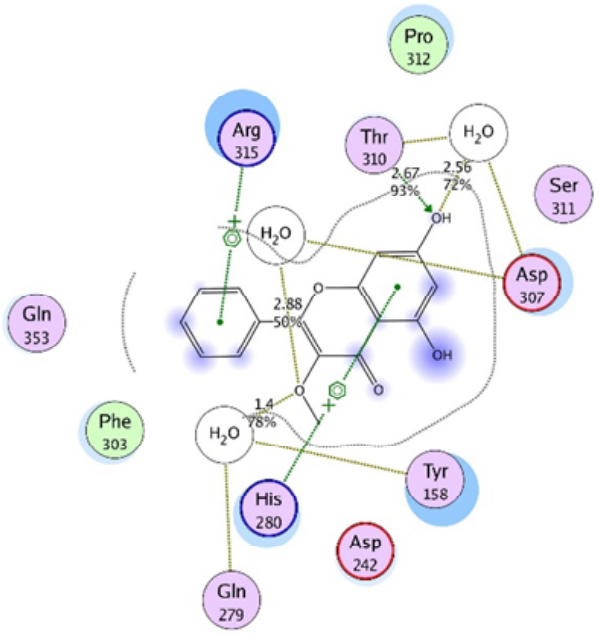


Figure 3: Interactions observed for the galangin 2 with the protein 3A4A

0.713 μM). While the compound zerumbone exhibited the better IC_{50} value against the nitric oxide radicals (with an IC_{50} value of 97.876 ± 0.853 μM). The structure-activity relationship of the compound is a considerable feature here in which each compound may be involved in donating electrons and thereby stabilising the radical group, which is one most suitable. In the table given below (Table 2), it is clear that, most of the isolated compounds exhibit better antioxidant properties, especially against the nitric oxide radicals, for which the standard gallic acid demonstrated an IC_{50} value of 320.0 ± 0.27 μM.

In silico antidiabetic and anticancer studies

Molecular docking was carried out by studying the protein-ligand interactions observed using LigPlot to find possible conformations and orientations for the ligands at the binding site. Here, the major compounds isolated from *Z. nimmonii* subjected to docking studies exhibited better antidiabetic properties than anticancer effects (Table 3). From the docking score values, it was found that compounds having more phenolic hydroxyl groups namely compounds **1**, **2** (galangin derivatives) and compound **7** (flavokavain B) could effectively bind to the targeted proteins with high docking score values.

The compounds **1** and **2**, which showed more activity towards both DPPH and ABTS scavenging assays, were effectively bound to the active sites of the enzymes with a docking score of -11.2018 kcal/mol and -11.2703 kcal/mol, respectively for the enzyme 2ITW and, -13.1593 kcal/mol and -11.9667 kcal/mol respectively, for the enzyme 3A4A. Compound **7**, which showed more activity on DPPH radical scavenging, was effectively bound to the active sites of the enzymes with a docking score of -9.84695 kcal/mol for the enzyme 2ITW and, -11.4503kcal/mol for the enzyme 3A4A.The compound having the highest docking score value towards the enzyme 3A4A was chosen for illustrating its effective binding interactions (Figure 3); three prominent interactions were observed for compound **1** (Galangin 1) with the antidiabetic enzyme 3A4A, which included two arene-cation interactions between the benzene ring and the basic amino acid residues Arg315 and His280, and one hydrogen bond acceptor interaction between the hydroxyl group at the seventh position with the hydrophilic amino acid residue Thr310. It is planned to study the effective enzymatic actions of the remaining compounds in future. Thus, the preliminary observations of docking studies support the need for the basis of antioxidative studies prior to other biological activity measurements.

CONCLUSION

In the present study, we have documented the detailed chemo-profiling, the first-time report by NMR characterisation, of the rhizomes of one of the *Zingiberaceae* plant species *Zingiber nimmonii*, and led to the isolation of eight different compounds. The preliminary extract level, and then the molecular level antioxidative studies suggest that, the consumption of this herb can effectively scavenge the undesirable free radicals produced in our body through various metabolic pathways. The docking studies of the isolated compounds support the structure activity relationships. These compounds

can play a vital role in modulating the antidiabetic and anticancer enzymes, and hence further studies are needed for understanding their mechanisms.

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