

BJP

Bangladesh Journal of Pharmacology

Research Article

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***In silico* analysis of compounds characterized from ethanolic extract of *Cucurbita pepo* with NF- κ B-inhibitory potential**

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Article Info

Received: 11 September 2014

Accepted: 19 October 2014

Available Online: 27 October 2014

DOI: 10.3329/bjp.v9i4.20420

Cite this article:

Rotimi SO, Rotimi OA, Obembe OO. *In silico* analysis of compounds characterized from ethanolic extract of *Cucurbita pepo* with NF- κ B-inhibitory potential. Bangladesh J Pharmacol. 2014; 9: 551-56.

Abstract

NF- κ B controls cellular growth properties of cells and its regulation is key to the management of disease like cancer. Although plant-derived bioactives have been reported to inhibit NF- κ B, there is limited knowledge on the interactions between the phytochemicals and NF- κ B. In this study, we identified the phytochemicals in ethanolic extract of *Cucurbita pepo* using Gas Chromatography-Mass Spectroscopy technique and used *in silico* approach to understand the interaction between the identified phytochemicals and NF- κ B using Molegro Virtual Docker. The docking algorithm showed that nine phytochemicals fit well into the pocket on NF- κ B. Our analysis showed that Lys144 is a prominent residue by involving in the binding of 9-octadecenoic acid (Z)-, methyl ester, hexadecanoic acid, methyl ester and octadecanoic acid, methyl ester with the moldock score of -55.5264, -57.4634 and -61.1258 respectively. Hence, the binding of these phytochemicals to NF- κ B could be responsible for the anti-inflammatory and anti-cancer property of *C. pepo*.

Introduction

Nuclear factor of kappa B (NF- κ B) regulates host inflammatory and immune responses and cellular growth properties by increasing the expression of cytokines and chemokines, the receptors involved in immune recognition. Cytokines that are stimulated by NF- κ B, such as IL-1 β and TNF- α , also directly activate the NF- κ B pathway, thus establishing a positive autoregulatory loop that can amplify the inflammatory response. In addition, NF- κ B is also involved in protecting cells from undergoing apoptosis in response to DNA damage or cytokine treatment resulting in the emergence of neoplasms (Karin et al., 2002).

Tumor cells in which NF- κ B is constitutively active are highly resistant to anticancer drugs and inhibition of NF- κ B activity in these cells greatly increases their sensitivity to such treatments (Karin et al., 2002). Hence, NF- κ B is now recognized as an important target for treatment of cancer and many inflammatory diseases. Thus, attention has been paid in the last decade to the

identification of compounds that can suppress the activity of NF- κ B and activation of its pathway.

Plants are rich in bioactive chemicals that make them to serve as therapeutics and valuable in the treatment of disease like cancer and inflammation (Yamamoto and Gaynor, 2001; Fadeyi et al., 2013). One of such plants is *Cucurbita pepo* (Cucurbitaceae). It is commonly known as "pumpkin" and distributed in different regions of world. It has been employed in folk medicine to treat benign prostatic hypertrophy (Shokrzadeh et al., 2010).

This study, therefore, investigated the molecular interaction between the phytochemicals in the ethanolic extract of *C. pepo* and the human NF- κ B.

Materials and Methods

Plant material

The leaves of *C. pepo* were collected from a local farm in Ilesa, Osun State, Nigeria and identification was done



by morphological characteristics. These were then oven dried completely at 27 to 30°C for 1 week. The dried leaves were ground to fine powder using a local grinder. 50 g of the ground leaves was macerated in 500 mL of absolute ethanol and kept in orbital shaker for 48 hours. The extract obtained was evaporated to dryness using rotary evaporator and reconstituted to 1 mg/mL and used for gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis

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 (99.9%) as a carrier gas at a constant flow rate of 22 cm/sec. One micron of extract 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.

Molecular docking

The SD files of the structures of the identified compounds were downloaded PubChem ([http://pubchem.](http://pubchem.ncbi.nlm.nih.gov/)

[ncbi.nlm.nih.gov/](http://pubchem.ncbi.nlm.nih.gov/)) and used as ligands. The 3D structure of p50 dimer (chain A and B) of human NF-κB was retrieved from Protein Data Bank (PDB: 1NFK) and used for this study.

The docking of the ligands with NF-κB was done on the Molegro Virtual Docker (MVD) (Thomsen and Christensen, 2006). MVD was first used to prepare the structure of the ligands and protein. MVD uses the molecular docking algorithm called MolDock, which is based on a novel search algorithm that combines differential evolution with a cavity prediction algorithm, to determine the potential binding site of the target protein and ligands. The scoring scheme ('dock score' refers to the approximate binding energies between protein and ligand, generally expressed in kcal/mol) was derived from Piecewise Linear Potential scoring functions (Thomsen and Christensen, 2006; Ajay Babu et al., 2012).

Results and Discussion

The result of the GC-MS analysis shows the various compounds present in the leaves of *C. pepo* (Table I and Figure 1). At least, 14 peaks were identified by mass spectrometry from the extracts and the identities of the compounds are listed in Table I. The most abundant of the phytochemicals are the octadecadienoic acid derivatives with retention time (min) 27.9, 29.4, 29.5, 30.4, 30.5 and 33.5. These compounds, as well as other structurally similar compounds like hexadecanoic acid, methyl stearate and phytol, have been reported to be present in the ethanolic extracts of other anti-inflammatory plants (Aparna et al., 2012; Ezhilan and Neelamegam, 2012; Saeed et al., 2012; Marimuthu et al., 2013; Silva et al., 2014). Silva et al (2014) reported that

Table I

Phytochemical components identified in the ethanolic extract of *C. pepo* leaves by GC-MS showing their RT, area, molecular weight, X log P, number of H-bond donor and number of H-bond acceptor

Sl. No	RT	Area	Compound name	Molecular weight	X logP	H- Bond donor	H- Bond acceptor
1	25.637	1.16	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	138.24992	3.9	0	0
2	26.976	3.20	Hexadecanoic acid, methyl ester	270.45066	7.9	0	2
3	27.977	0.85	Octadecanoic acid, ethyl ester	312.5304	8.9	0	2
4	29.471	2.64	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294.47206	6.9	0	2
5	29.545	8.08	9-Octadecenoic acid (Z)-, methyl ester	296.48794	7.6	0	2
6	29.763	3.24	Phytol	296.531	8.2	1	1
7	29.871	1.67	Methyl stearate	298.50382	9.0	0	2
8	30.495	10.50	9,12-Octadecadienoic acid (Z,Z)-	280.44548	6.8	1	2
9	30.587	1.60	11-Octadecenoic acid, (Z)-	282.46136	6.5	1	2
10	30.632	1.52	9-Oxabicyclo[6.1.0]nonane, cis-	126.19616	2.3	0	1
11	31.348	8.78	Cholest-4-en-3-one	384.63766	8.4	0	1
12	31.382	4.24	Cholest-5-en-3-one	384.63766	8.2	0	1
13	33.545	1.57	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	356.5399	6.5	2	4
14	34.764	1.73	Oxirane, tetradecyl-	240.42468	7.3	0	1

Table II					
Docking results showing MolDock score, H-bond energy, number of H-bond and H-bond donor					
S/N	Ligand	MolDock score	H-Bond	Number of H-bond	H-bond donor
1	9-Octadecenoic acid (Z)-, methyl ester	-55.5264	-1.32052	1	Lys144
2	9-Oxabicyclo[6.1.0]nonane, cis-	-2.66845	-3.29281	2	Lys275 Gln306
3	11-Octadecenoic acid, (Z)-	-80.1009	-1.51686	1	Lys272
4	Hexadecanoic acid, methyl ester	-57.4634	-2.16961	1	Lys144
5	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	-35.9643	-9.65187	4	Arg54 Glu60
6	Octadecanoic acid, methyl ester	-61.1258	-4.24413	2	Lys144 Leu207
7	Phytol	-41.2086	-3.00898	3	His141 Phe55 Asp239
8	Oxirane, tetradecyl-	-87.0015	-2.17092	1	Arg54
9	cis-1,2-Epoxyoctane	-53.1884	-1.52727		Arg54

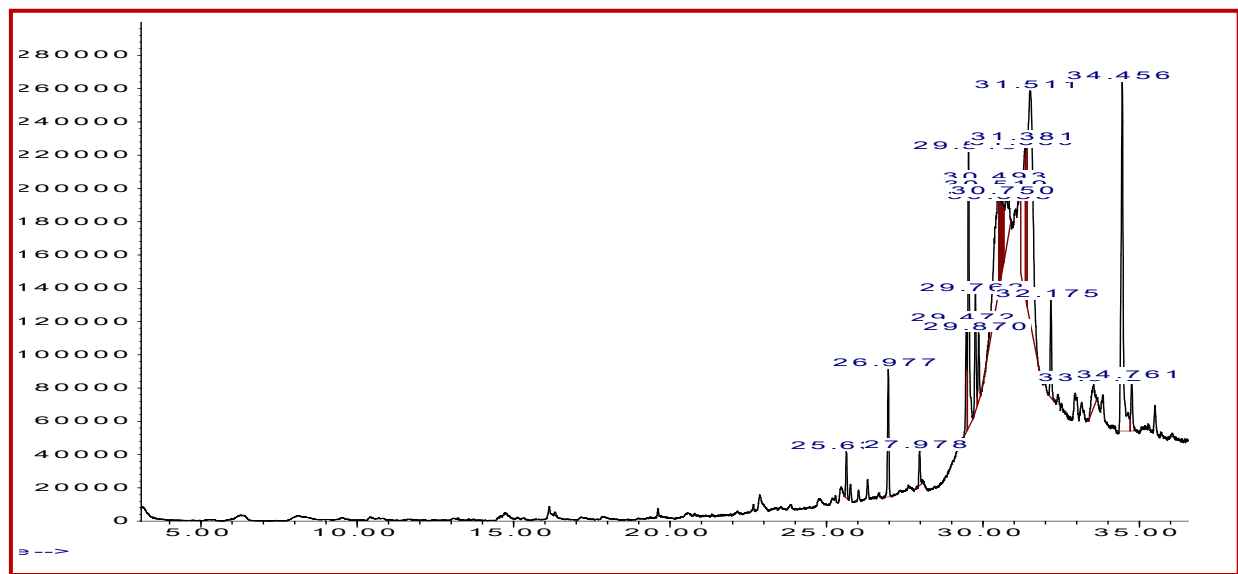


Figure 1: GC-MS chromatogram of ethanolic extract of *C. pepo* leaves

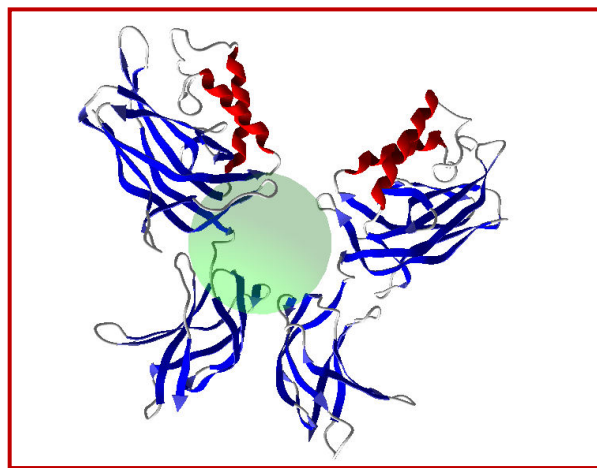


Figure 2: Stereoview of the NF-kappaB p50 dimer chain A and B. The binding pocket is highlighted in green

phytol inhibits inflammation by reducing cytokine production and oxidative stress while Saeed (2012) reported that the derivatives of hexadecanoic acid prevented lipopolysaccharide-induced inflammation by suppressing the production of cytokines and NF- κ B. The anti-inflammatory property of this compound has been linked to their structures (Aparna et al., 2012).

The molecular docking results showed that some of these phytochemicals can also directly bind to and inhibit the activity of NF- κ B (Figure 2). Figure 3A-I shows the binding of the phytochemicals into the binding pocket (Figure 2) of the human NF- κ B. This binding is stabilized by hydrogens (H) bond(s) between some amino acid(s) present within the binding pocket of NF- κ B and H-bond acceptors on the ligands. The MolDock scores and the atoms involved in the bond

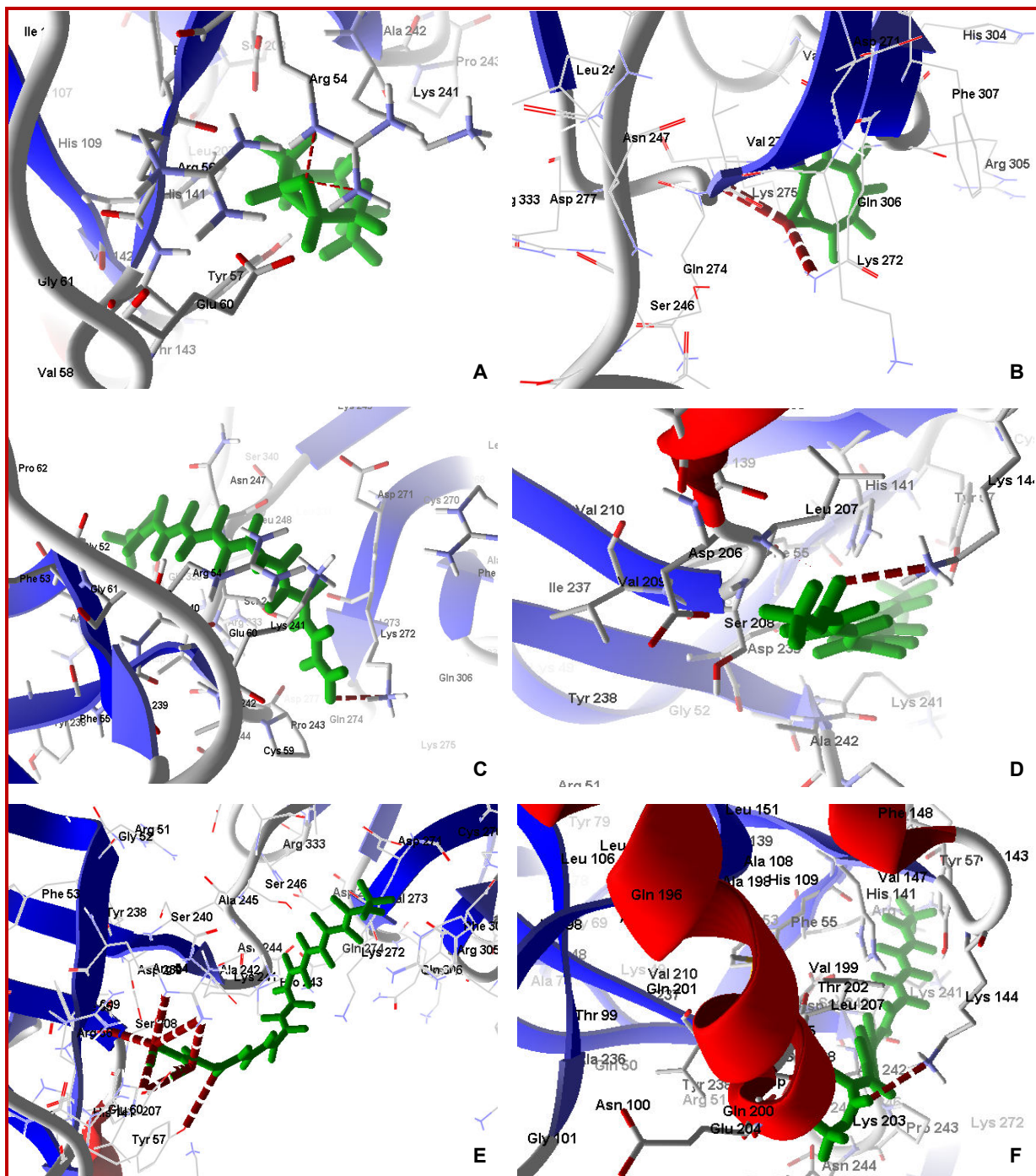


Figure 3: Stereo view of the binding of ligands to NF-kappa B. The ligands are highlighted in green. (A) cis-1,2-Epoxyoctadecanoic acid; (B) 9-Oxabicyclo[6.1.0]nonane, cis-; (C) 11-Octadecenoic acid, (Z)- (D) Hexadecanoic acid, methyl ester; (E) 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester; (F) 9-Octadecenoic acid (Z)-, methyl ester; (G) Phytol; (H) Octadecanoic acid, methyl ester; (I) Oxirane, tetradecyl-

formation are shown in Table II with oxirane, tetradecyl- having the best MolDock score of -87.0015 and followed by 11-Octadecenoic acid, (Z)- with MolDock score of -80.1009. Studies have shown that the plasma level of this omega-7 fatty acid has a negative correlation with inflammation (Block et al., 2012; Djoussé et al.,

2014). The H-bond donors are majorly the ϵ nitrogen of Lys and the guanidine nitrogen of Arg. For the binding of 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester the ϵ nitrogen of Arg was also involved in the formation of the H-bond. Other amino acids involved in the formation of H-bond include Leu for octadecanoic

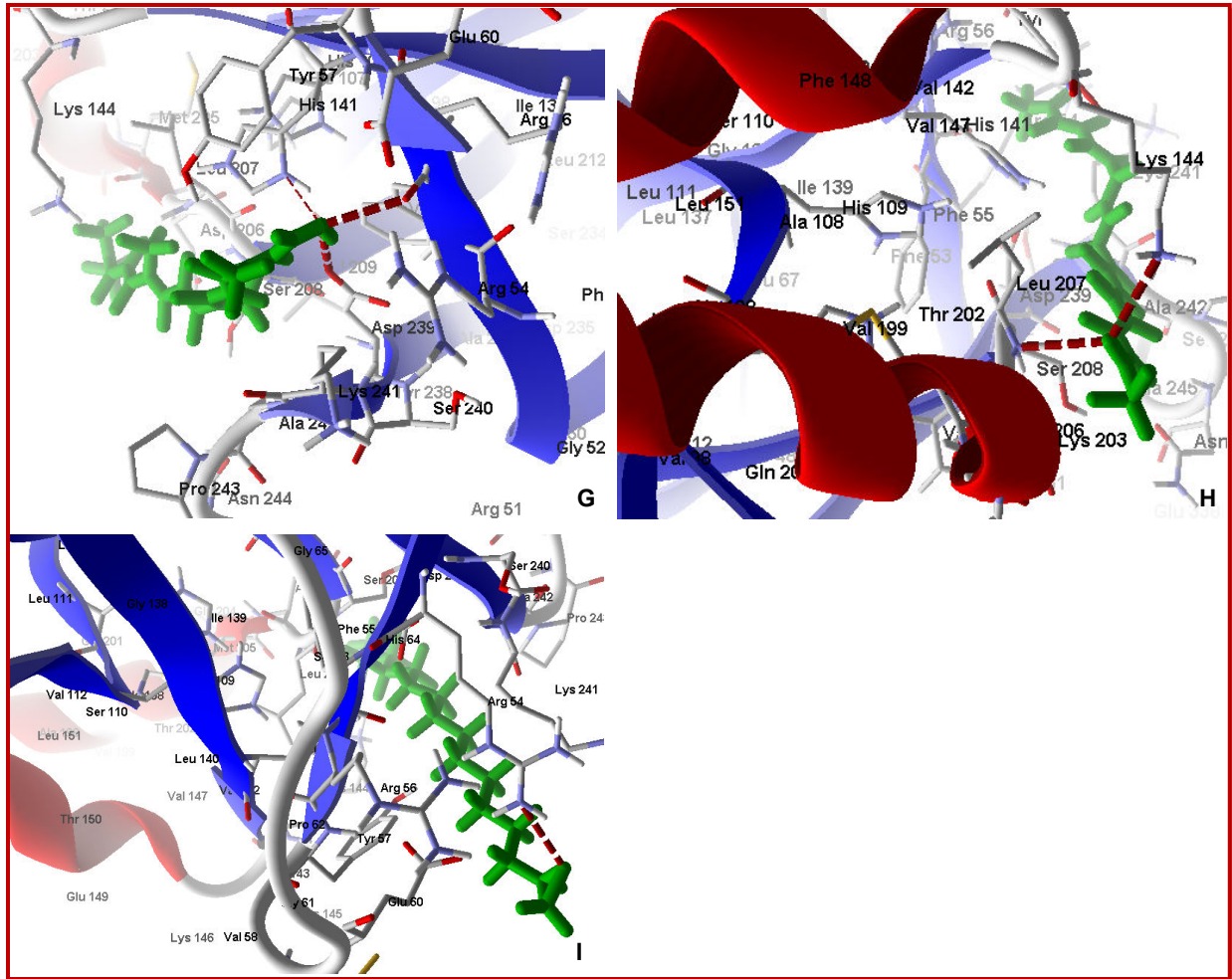


Figure 3: Stereo view of the binding of ligands to NF-kappa B. The ligands are highlighted in green. (A) cis-1,2-Epoxy cyclooctane; (B) 9-Oxabicyclo[6.1.0]nonane, cis-; (C) 11-Octadecenoic acid, (Z)- (D) Hexadecanoic acid, methyl ester; (E) 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester; (F) 9-Octadecenoic acid (Z)-, methyl ester; (G) Phytol; (H) Octadecanoic acid, methyl ester; (I) Oxirane, tetradecyl-. (Continued)

acid, methyl ester, His and Asp for phytol, Glu for 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester as well as Gln for 9-oxabicyclo[6.1.0]nonane, cis-.

Several plant-derived bioactives have been reported as potential inhibitors of NF- κ B by interacting with the enzyme directly (Nam, 2006). Using *in silico* and *in vitro* techniques Piccagli et al. (2008) reported similar binding of bioactives from medicinal plants against NF- κ B and concluded that the docking result is predictive of biochemical activity.

Our finding therefore suggests that these phytochemicals could account for account the anti-inflammatory and anti-cancer property of *C. pepo*.

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