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Characterization of the compound of longan honey from indonesia using LC-MS/MS and FTIR and the mechanism of inhibition of HEp-2 cells

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ABSTRACT: Indonesian honey contains active compounds that have the potential as antioxidant and anticancer, especially as anticancer of the larynx through inhibition of HEp-2 cells. This study aims to determine the active compounds in longan honey and proposes the mechanism of action in inhibiting HEp-2 cells. The sample was used in the form of longan obtained from honey bee breeders in Central Java. Honey samples were extracted using methanol, and then liquid-liquid partitioning was carried out successively using n-hexane and ethyl acetate. Isolation and characterization of longan honey samples using FTIR and LC-MS/MS showed the presence of the following compounds: Xanthoxol glycosides, Santonin, Octadecanamide, Indole-3 carboxylaldehyde, 3,4-dimethoxycinnamic acid, Dimethyl esculetin, Tryptophan, O-acetyl-L- serine, D-glucose-6-phosphate, Feruloiltyramine, Lauryl diethanolamide, Taurine, 6-mercaptopurine, 3-(2,4-dichlorophenyl)-4-phenylcoumarin, 3',4'-dimethoxy-3-hydroxy-6-methylflavone and D-1-((3-carboxypropyl)amino)-1-deoxyfructose. The compounds in longan honey against HEp-2 cells is quercetin, 3,6-dimethoxycinnamic acid, phenyl coumarin, dimethyl esculetin, santonin, 6-mercaptopurine, and feruloyltyramine. The proposed mechanism of active compound in honey to inhibit HEp-2 cells in several ways including via caspase pathway and purine synthesis and other relevant mechanisms. However, this assumption needs to be tested further to obtain more precise information regarding the mechanism of inhibition of HEp-2 cells.

KEYWORDS: HEp-2 Cells; inhibition; longan honey; LC-MS/MS; FTIR.

1. INTRODUCTION

Honey is a sweet, viscous liquid that honey bees make from a variety of floral nectar sources. More than 200 different substances can be found in natural honey, the majority of which are water, enzymes, carbohydrates (75% consisting of monosaccharides like glucose and fructose and 15% of which are disaccharides like sucrose and maltose), vitamins, minerals, phenolics and flavonoids, and natural pigments [1]. Each honey has a unique composition that is affected by the type of bee, habitat, flower, and region from where the honey is produced. [2]. Investigations into the contents of honey from Indonesia and throughout the world have revealed the existence of a number of secondary metabolites, including beta carotene [3], vitamins A and E [4], and C [5], as well as flavonoids and phenolics. Apigenin (0.03 mg/100 g), isorhamnetin (0.06 mg/100 g), kaempferol (0.06 mg/100 g), luteolin (0.28 mg/100 g), quercetin (0.31 mg/100 g), and myricetin (0.36 mg/100 g) are all present in mixed realities of honey samples from different nations [6]. As with Ziziphus honey (ZH) from the Hail area of Saudi Arabia, other varieties of honey in Indonesia have also been studied for a variety of properties, including activities of antibacterial [7], antioxidant [8], and potential as an anticancer [9].

According to early research by Sumarlin et al., the longan honey ethyl acetate extract has a 65.18% inhibitory effect at 100 ppm on HEp-2 longan cells [10]. Further research is required to determine which substances have this inhibitory capacity and how they perform. The Extract characterization method can be used to characterize the suspected compounds. The active fraction of honey was analyzed using a Fourier

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Transform Infra-Red (FTIR) Spectrophotometer and a Liquid Chromatography Spectrophotometer Mass Tandem Spectrophotometer Mass (LC-MS/MS).

2. RESULTS AND DISCUSSION

2.1. Extraction and Liquid-Liquid Partitions in Longan Honey

In this study, the extract yield was calculated by comparing the sample's initial weight with the obtained extract (Table 1). The results showed that the yield of methanol extract was 89.21% after the liquid-liquid partitioning of ethyl acetate extract was lower than that of polar or water extracts. The difference is that most honey components generally consist of sugar 61.88%, which is soluble in polar solvents or water [11]. The water content in honey also increases the yield of polar extracts.

Table 1. Results of methanol extraction and liquid-liquid partition of longan honey

Sample	Methanol extract weight(g)	Yield (% b/b)	Solvent	Extract weight (g)	Yield (% b/b)
Longan Honey	89.23	89.21	N-hexane	0	0
			Ethyl Acetate	28.34	28.33
			Water	60.57	60.55

2.2. Extract Longan Honey of Ethyl Acetate Fractionation

The results of research by Sumarlin et al. [10] on the liquid-liquid partition fraction N-hexane, acetyl acetate of longan honey showed the most significant inhibitory activity (%) of HEp-2 cell proliferation in the ethyl acetate fraction of 65.18% at a concentration of 100 ppm. The ethyl acetate fraction with the most significant inhibition was separated in this study by the thin layer chromatography (TLC) method using a Silica gel 60 F254. Spots on the TLC plate dotted with ethyl acetate extract of longan honey were then eluted and monitored under a UV lamp at 254 nm & 365 nm. The results of the TLC separation started from N-hexane : ethyl acetate with a ratio of 3:7 to N-hexane : ethyl acetate : acetone with a ratio of 1:3:1. The best separation occurs when using n-hexane: ethyl acetate : acetone in a ratio of 1:3:1 and at a wavelength of 254 nm, three spots are found (Figure 1a, bottom).

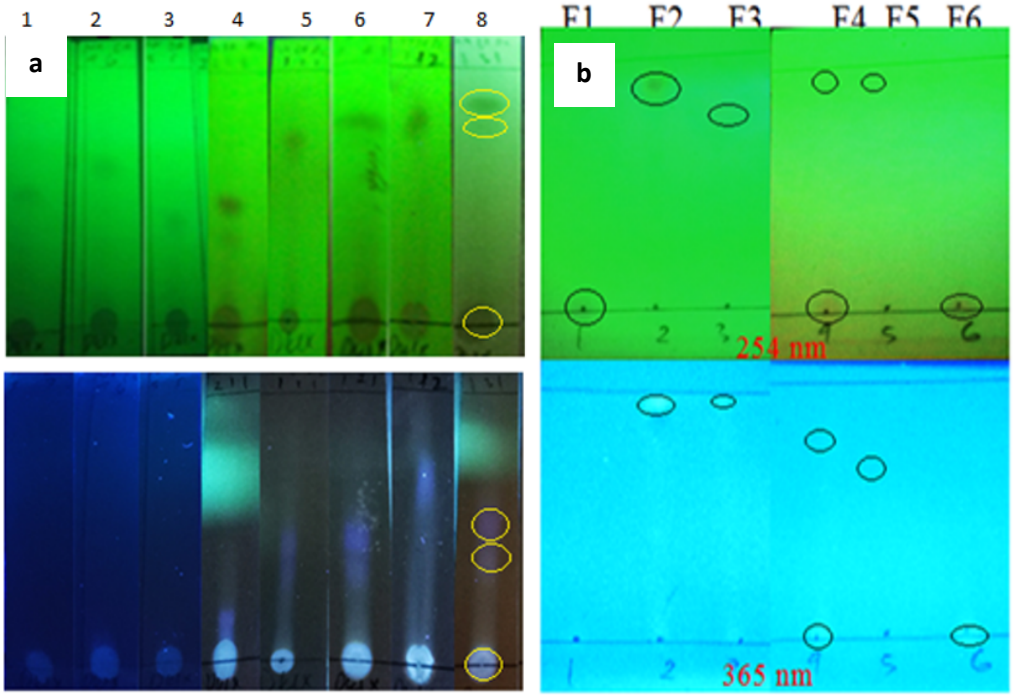

Figure 1. a. Separating the ethyl acetate extract fraction using TLC, b. The results of the fractionation of longan honey ethyl acetate extract

The TLC separation results (Figure 1a) were separated again by the gravitational column chromatography (KGV) method using silica gel 60 (Merck) stationary phase with a size of 70-230 mesh, and the mobile phase used was the mobile phase which gave the best separation in TLC. The fraction resulting from column chromatography separation was accommodated in vials and re-identified by the TLC method based on the similarity of the spots produced. The end result is six fractions with masses F1 – F6, respectively 1.6 g, 1.21 g, 2.01 g, 1.8 g, 2.6 g, and 4.06 g (Figure 1b).

2.3. Characteristics of Ethyl Acetate Extract and Longan Honey Fraction

The ethyl acetate fraction and extract of longan honey were further characterized using FTIR instruments (Bruker Inc.) and LC-MS/MS (Waters, USA) (Table 8 and Table 9) to determine functional groups and suspected active compounds capable of inhibiting HEp-2 cell proliferation. Analysis of suspected compounds was carried out by comparing the presence of functional groups and the similarity of the mass spectra of the sample with the mass spectra of a compound from the Mass Bank online database, the Human Metabolite Database (HMDB), and the Mass Bank of North America.

Table 2. Results of FTIR functional group analysis of ethyl acetate extract and longan honey fraction

Wave Number (cm^{-1})	Wave Number (referensi)	Fungsional Group	Sample
3396-3305	3600-3200	-OH	Extract EA, F3, F4, F5, and F6
2964-2836	2970-2850	CH aliphatic	F1, F2, F4, and F5
1661-1421	1680-1475	C=C aromatic	Extract EA, F3, F4, F5, and F6
1260-1257	1270-1230	C-O-C	F1, F2, F4, and F6
1062-1017	1300-1000	C-O	Extract EA, F3, F4, F5, and F6

The IR spectrum of longan honey ethyl acetate extract shows the presence of a functional group of a compound based on absorption in the IR region, the wide and strong band at wave number 3396 cm^{-1} indicates the presence of –OH groups (3400-3300 cm^{-1}) which experience a broadening of the peak due to hydrogen bonding and strengthened by C-O absorption (1300-1000 cm^{-1}) at wave number 1259-1062 cm^{-1}. The absorption at wave numbers 1647 cm^{-1} and 1454 cm^{-1} is caused by the aromatic C=C group (Figure 2, Table 2). The IR spectra analysis showed that the ethyl acetate extract of longan honey has functional groups -OH, C-O, and aromatic C=C. Based on IR spectra and phytochemical tests, it is suspected that the ethyl acetate extract of longan honey contains flavonoid compounds.

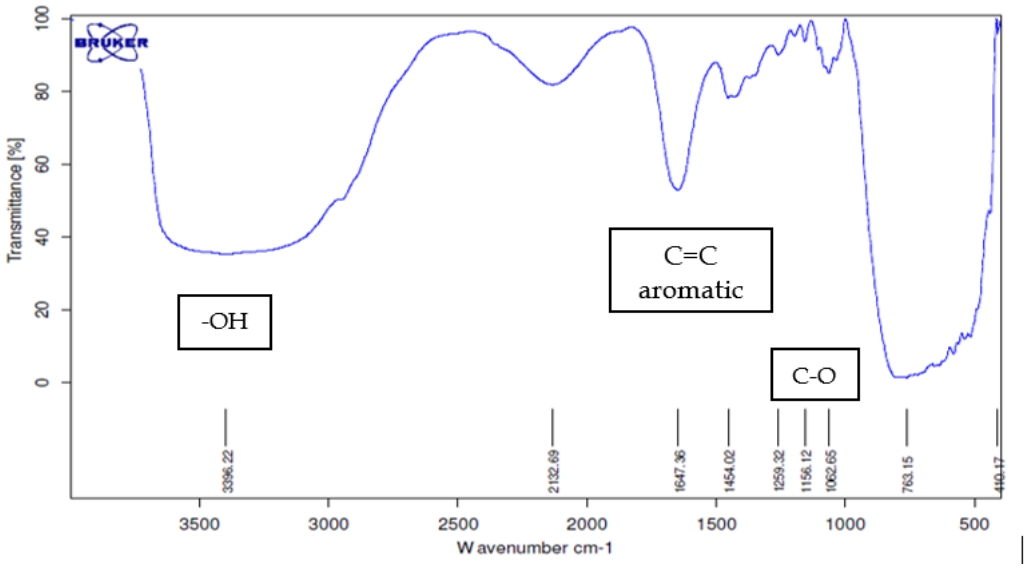

Figure 2. IR spectra of ethyl acetate extract of longan honey

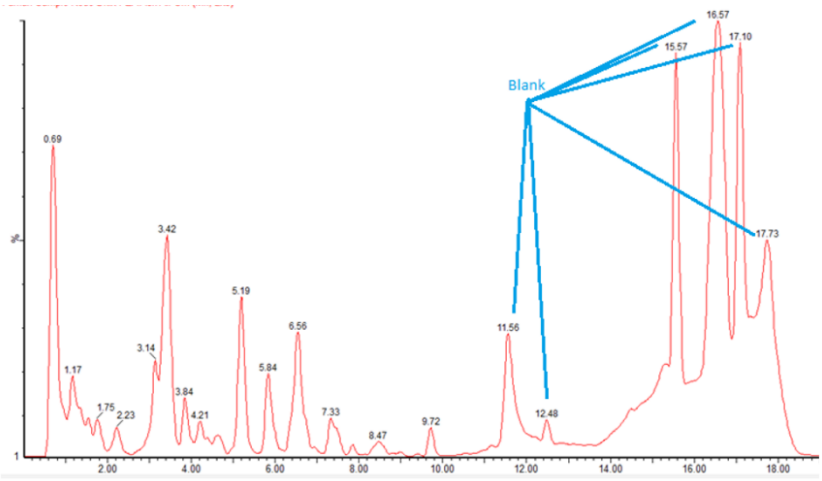

Figure 3. Chromatogram of longan honey ethyl acetate extract

The results of the FTIR analysis were then confirmed again with the LC-MS/MS test. The mass spectra of LC-MS/MS analysis using Mass Lynx software (version 4.1) were then compared with the online databases Mass Bank, HMDB, and Mass Bank of North America (MoNA). The results of the analysis of longan honey ethyl acetate extract using LC-MS/MS produced chromatograms with different retention times (Figure 3). The chromatogram of longan honey ethyl acetate extract showed 20 chromatogram peaks with six blank peaks, and 11 peaks were identified (Table 3).

Table 3. Presumptive compounds in ethyl acetate extract of longan honey

Rt	$[M+H]^+$ observation	$[M+H]^+$ reference	Potential Compounds
0.69	365.1058	365.0867	Xanthotoxol glycosides
1.75	194.1177	194.0812	Phenyl acetyl glycine
3.14	303.1072	303.0656	Quercetin
3.42	247.1326	247.1331	Santonin
3.84	207.1380	207.1749	4-(1,1,3,3-Tetramethylbutyl)-phenol
4.21	146.0601	146.0606	Indole-3-carboxylaldehyde
5.19	201.1016	201.1022	Harmalol
5.84	207.1383	207.0650	Dimethyl esculetin
6.56	261.1486	261.1001	D-glucose-6-phosphate
7.33	242.1544	242.1539	Dehydroisocalciporone
9.72	288.2535	288.2533	Lauryl diethanolamide

Each fraction 1-6 (Figure 1b) was identified using LC-MS/MS which also showed that the ethyl acetate extract and most of the fractions contained xanthosol glycoside compounds. In addition, this analysis also shows that longan honey also contains other compounds such as amino acids and carbohydrates, namely O-acetyl-L-serine, tryptophan, D-glucose-6-phosphate, and isomaltose (Table 4).

Table 4. Compounds suspected in the fraction 1-6 longan honey

Fraction	Rt	$[M+H]^+$ observation	$[M+H]^+$ reference	Potential Compounds
1	0.69	365.1058	365.1058	Xanthosol glycosides
	3.52	247.1326	247.1331	Santonin
	12.63	284.3318	284.2953	Octadecanamide
2	4.28	146.0603	146.0606	Indole-3-carboxylaldehyde
	5.53	209.1543	209.0736	3,4-dimethoxycinnamic acid
	5.92	207.1385	207.0650	Dimethyl esculetin
	6.46	205.1226	205.0949	tryptophan
3	0.69	365.105	365.1058	Xanthosol glycosides
	1.41	127.0394	127.0015	Imidazole-4-acetate
	2.28	148.0752	148.0532	O-acetyl-L-serine
	3.56	146.0603	146.0606	Indole-3-carboxylaldehyde
	3.9	207.139	207.0650	Dimethyl esculetin
	4.26	261.1494	261.3001	D-glucose-6-phosphate
	4.8	314.1756	314.1314	Feruloiltyramine
	5.53	288.2536	288.2533	Lauryl diethanolamide
4	0.69	365.1056	365.1058	Xanthosol glycosides
	1.03	126.0551	126.0255	taurine
	2.28	148.0754	148.0532	O-acetyl-L-serine
	3.42	153.1278	153.3157	6-mercaptopurine
	5.10	288.2553	288.2533	Lauryl diethanolamide
5	0.69	365.1058	365.1058	Xanthosol glycosides
	1.02	126.0553	126.0255	taurine
	2.27	146.0602	146.0606	Indole-3-carboxylaldehyde
	3.47	247.1326	247.1331	Santonin
	4.43	314.1234	314.1314	Feruloiltyramine
	9.56	367.2459	367.0267	3(2,4-dichlorophenyl)-4-phenylcoumarin
	14.02	313,2748	313.1776	3',4'-dimethoxy-3-hydroxy-6-methylflavone
6	0.69	365.1078	365.1058	Xanthosol glycosides
	3.34	266.1021	266.1240	D-1-((3-carboxypropyl)amino)-1-deoxyfructose

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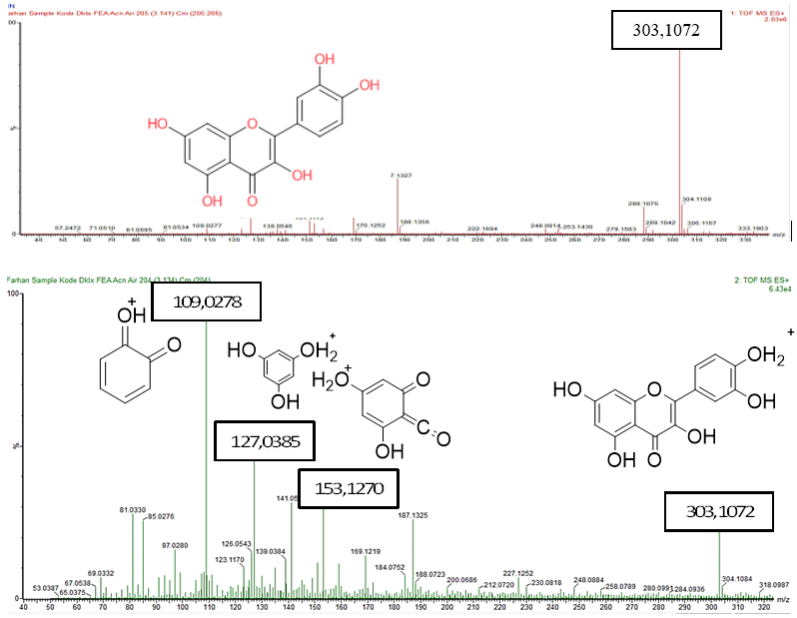

Figure 4. Mass spectra of suspected quercetin compounds

The mass spectra at a retention time of 3.14 show the m/z value with the most abundance (100%), namely 303.1073 and will be fragmented to become m/z 109.0278 (Figure 4). The ionization used is [M+H]+, so the actual m/z value is 302.1073, and the predicted molecular formula of the compound is $C_{15}H_{10}O_7$. The m/z weight of 303.1073 is also called the parent ion, while the m/z 109.0278 is the second daughter ion. These values can be used to predict a compound. When compared with Mass Bank's online database, there are some similarities in the mass spectra of the measurements results with the mass spectra of quercetin, namely at m/z 303; 153; 127, 109, and based on the results of the phytochemical and FTIR tests which showed the presence of compounds belonging to the flavonoid class, it is presumed that the compound that appeared at a retention time of 3.14 was a flavonoid compound, namely quercetin (Figure 4.). Other spectra were found in this study (Figure 5 and Figure 6).

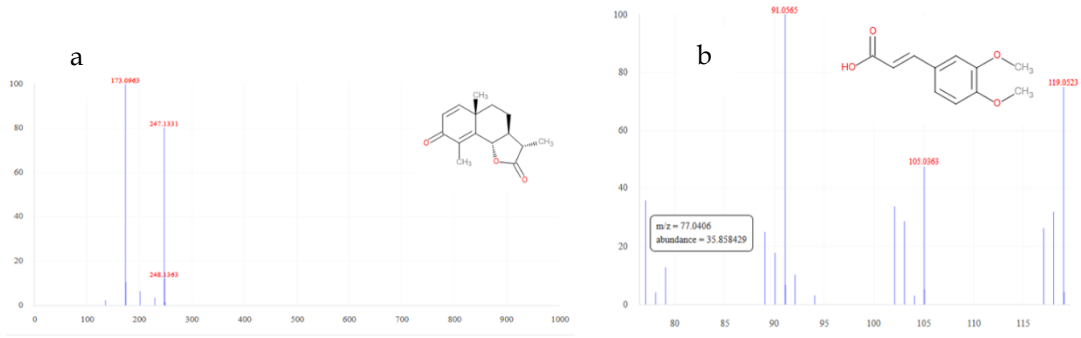

Figure 5. Mass spectrum of compound suspected, (a). Santonin, (b). 3,4-dimethoxycinnamic acid

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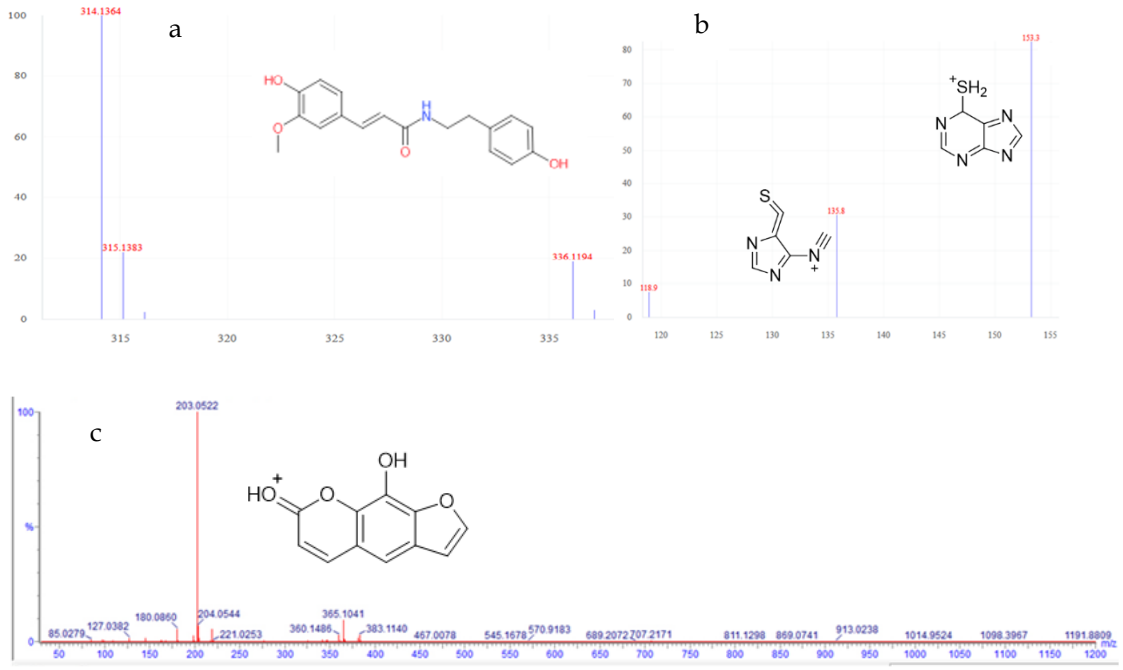


Figure 6. Mass spectrum of compound suspected, (a). feruloiltiramin, (b). 6-mercaptopurine, (c). xanthosol glycosides

2.4. Alleged Mechanism of Inhibition of HEp-2 Cells by Honey

Previous studies have shown that longan honey extract can inhibit the growth of HEp-2 cells by 65.18% at a concentration of 100 ppm [10]. The results of the analysis of the isolated compounds in this study indicated the alleged mechanism of action of the HEp-2 cell inhibition.

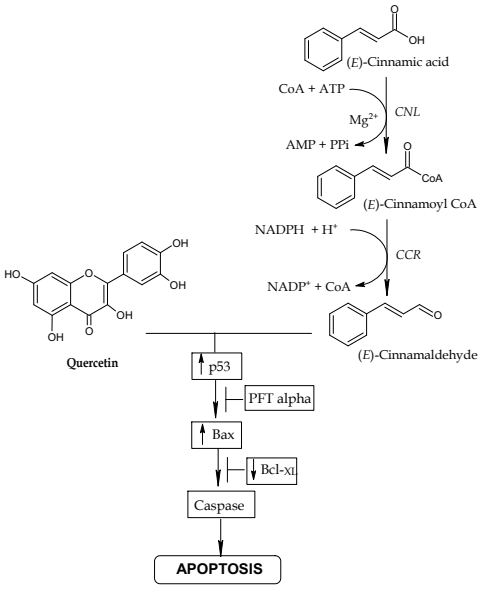


Figure 7. Regulation of apoptosis by quercetin and cinnamic acid [12]

Quercetin (Table 3) and cinnamic acid derivatives (3,4 dimethoxycinnamic acid) (Table 4) are thought to have an incredibly active role in inhibiting HEp-2 cancer cells. Quercetin is a flavonol compound and is found in various plants. This compound has been isolated from Dutch eggplant skins, beluntas leaves, and Sonneratia alba stem bark [13], [14], [15]. Quercetin has been shown to inhibit cancer cells in vitro, such as CT-

26 cells (colon cancer), prostate cancer PC3 cells, MCF-7 cells (breast cancer), Raji cells (lymph cancer), and CHO cells (ovarian cancer). In addition, in vivo, quercetin reduced the volume of MCF-7 and CT-26 tumor cells grown in mice [16].

Meanwhile, cinnamic acid, especially in the form of cinnamaldehyde, can increase the expression of p53, caspase-3, and bax, decrease the activity of the anti-apoptotic proteins bax-2 and pifithrin-α (PFT-α) which are p53 inhibitors [12]; [17]. Cinnamic acid is converted into the form of cinnamaldehyde through several stages. The first stage of cinnamic acid reacts with the enzyme cinnamic acid-CoA ligase (CNL) to form cinnamyl-CoA. Furthermore, cinnamoyl-CoA turns into the form of cinnamaldehyde with the help of the enzyme cinnamoyl-CoA reductase (Figure 7).

In another explanation, it was also stated that honey will activate p53 which will modulate the expression of pro-apoptotic proteins such as bax [18]. Honey and Aloe vera have been shown to be able to increase bax expression and suppress bcl-2 expression which causes Walker cancer cells to undergo apoptosis and death. Bax and bcl-2 are involved in the process of cell apoptosis, bax which has a pro-apoptotic effect can be inhibited by bcl-2 so that increased expression of bax and decreased expression of bcl-2 will trigger the process of apoptosis in cancer cells and increase the likelihood of cancer cells dying.

Compounds that also work through the mechanism of inducing apoptosis so that they are thought to inhibit the proliferation of HEp-2 cells are dimethyl esculetin and 3,4-dimethoxycinnamic acid (Table 4). Flavonoid and phenolic compounds in honey, such as cinnamic acid and coumaric acid are active as anti-leukemia by activating the 'arrest' cell cycle at the G0 stage and inducing cell apoptosis [19]. Dimethyl esculetin (Table 3) is also thought to be active as an anticancer because esculetin has been shown to actively inhibit pancreatic cancer cells by activating the G1 'arrest' cell cycle phase to carry out cell repair and induce apoptosis through activating caspase 3, 8, and 9 [20]. Dimethyl esculetin (Table 3) is also thought to be active as an anticancer because esculetin has been shown to actively inhibit pancreatic cancer cells by activating the G1 'arrest' cell cycle phase to carry out cell repair and induce apoptosis through activating caspase 3, 8, and 9 [20].

Honeys suppressed the production of pro-inflammatory markers NO, IL-1β and IL-6 induced by lipopolysaccharide and promoted the expression of anti-inflammatory cytokines IL-10 in RAW 264.7 cells [21]. Caspase 3 can trigger apoptosis by cleaving proteins in the cytoplasm and nucleus [18]. Sulfurofen isolated from methylene chloride extract of red cabbage (Brassica oleracea var rubra) was able to inhibit the growth of HEp-2 cells by 82.34% at a concentration of 500 ppm by increasing the expression of pro-apoptotic proteins (p53, bax, and caspase) and suppressing protein expression bcl-2 antiapoptosis [22].

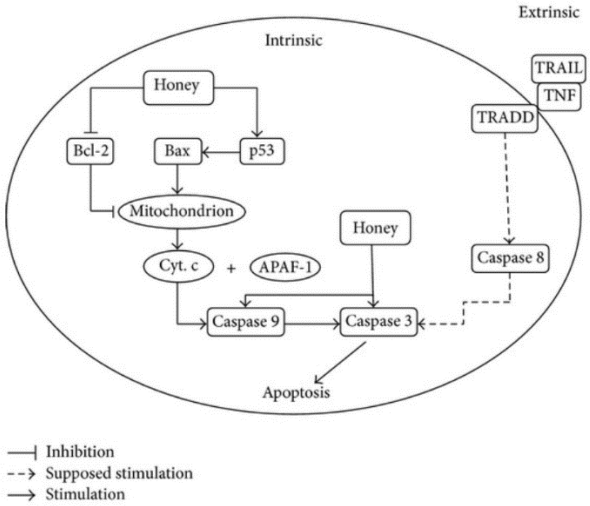


Figure 8. The process of regulating the mechanism of apoptosis by honey [18]

Honey regulation of cell apoptosis regulatory proteins is also an essential process in inhibiting cancer cells, including HEp-2 cells. The active compounds in honey will increase the expression of the p53 gene (tumor suppressor gene), which prevents cell division from occurring, thereby preventing tumor cells from developing. Expression of the p53 gene is triggered by DNA damage present in tumor cells but normal cells that do not experience DNA damage will still carry out cell division. The p53 protein causes GI arrest or apoptosis and stimulates pro-apoptotic proteins such as Bax. Bax bound to mitochondria will trigger the

release of pro-apoptotic factors, namely cytochrome C proteins and Apaf-1, into the cytoplasm, activating the caspase nine pathway.

Furthermore, caspase 9 will activate caspase 3 which will become the executor in the process of apoptosis. Caspase 3 will break down proteins in the cytoplasm and nucleus, causing the HEp-2 cell nucleus to break down. In addition, TNF is an extrinsic apoptotic mediator to initiate the caspase 8 activation pathway, which directly converts pro-caspase 3 to caspase 3 and then the caspase 3 pathway is activated. Mitochondrial proteins Bcl-2 and Bcl-xL can block the release of cytochrome c from mitochondria and bind Apaf-1, which prevents activation of caspase 9, so it is essential to reduce Bcl-2 protein so that the process of apoptosis continues (Figure 8) [18].

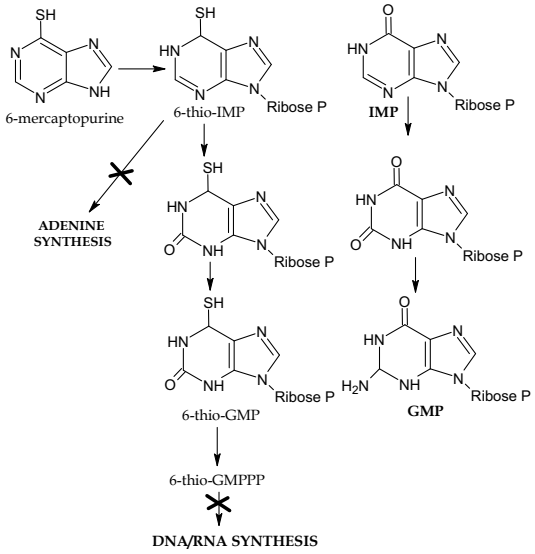


Figure 9. Mechanism of 6-mercaptopurine inhibition [23]

Another compound that is thought to have a role in inhibiting HEp-2 cells is 6-mercaptopurine (6-MP). The compound 6-MP present in fraction 4 (Table 4) is thought to play an active role in inhibiting the proliferation of HEp-2 cells (Figure 9). 6-MP is a class of purine base compounds such as hypoxanthine and adenine. 6-MP is the first active metabolite inhibitor shown to suppress cancer cells. 6-MP is a clinically important antitumor drug and its commercially available form is provided as monohydrate, belonging to biopharmaceuticals classification system (BCS) class II category. The combination of bismuth(III) (Bi(III)) with 6-MP was proved to significantly improve the anticancer activity of 6-MP [24]. 6-MP is also a purine antagonist that has long been used as an antileukemic drug, cancer treatment, and immunosuppressive agent.

Compound 6-MP has an inhibition mechanism through the purine synthesis pathway and can inhibit DNA/RNA synthesis [23]. The compounds in fraction 4 (Table 4) indicate other inhibition mechanisms that affect HEp-2 cell proliferation. The 6-MP compound turns into 6-thio-inosine monophosphate (6-TIMP) with the help of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HRTP'ase). 6-TIMP can inhibit de novo purine synthesis, especially adenosine, because the ketone group (-C=O) at position 6 is replaced by a thio group (-C-SH) so that it cannot bind to asparagine and HEp-2 cells cannot produce adenosine monophosphate (AMP). In addition, guanine can also undergo modification in the ketone group to form a thio group to form thio-guanosine monophosphate (TGMP). The absence of adenosine can cause DNA/RNA replication to not occur. As a result, HEp-2 cancer cells cannot carry out cell division (Figure 9).

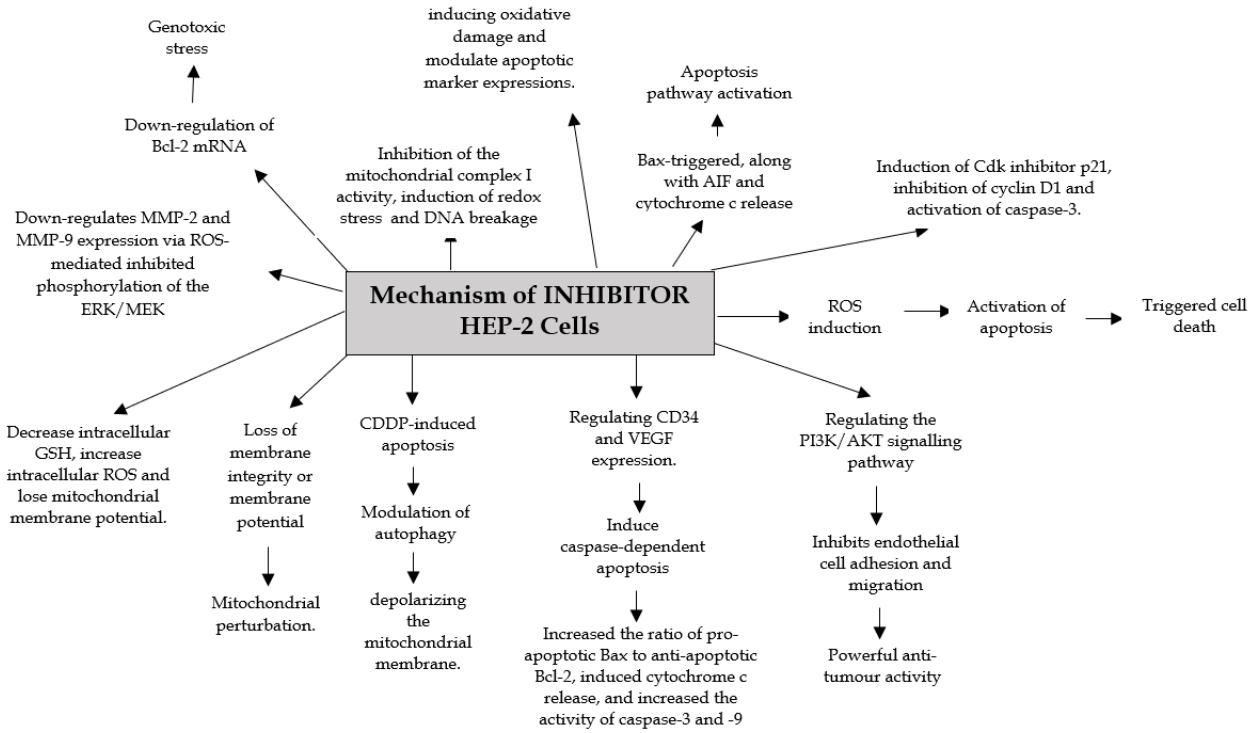


Figure 10. Multiple mechanisms of HEp-2 cell inhibition

In addition, there are many other mechanisms for inhibiting HEp-2 cells (Figure 10). This is possible because each sample in various studies contains different bioactive components (Table 5).

Table 5. Hep-2 cells and their mechanism of action in various materials

No	Source of HEp-2 Cells	Mechanism	Sample	References
1.	Human laryngeal cancer cells (HEp-2 line) and human non-tumor cells (HEK-293 line)	Inhibition of the mitochondrial complex I activity, induction of redox stress and DNA breakage. The apoptosis pathway activation occurs via Bax-triggered, along with AIF and cytochrome c release, and it is independent of p53 incitement	Extract obtained from A. *angustifolia*	[25]
2.	The HEp-2 human laryngeal squamous carcinoma cell line	Regulating CD34 and VEGF expression, induce caspase-dependent apoptosis, Regulating the PI3K/AKT signaling pathway, inhibits endothelial cell adhesion and migration, powerful anti-tumor activity	Lj-RGD4 is a novel toxin protein from the salivary gland of Lampetra japonica that is characterized by 4 RGD motifs.	[26]
3.	HEp-2 cells, considered to originate from a human laryngeal carcinoma	Stable knockdown of CK2α inhibited the aggressive migration and invasion of human laryngeal carcinoma cells and enhanced their sensitivity to chemotherapeutic drugs.	Short hairpin RNA (shRNA)-mediated RNA interference (RNAi) technology	[27]
4.	The HEp-2 human laryngocarcinoma cell line	Loss of mitochondrial membrane potential, increased the ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2, induced cytochrome c release, and increased the activity of caspase-3 and -9, which altogether account for apoptotic cell death.	The materials of L. *sordida* fruiting bodies were purchased from Harbin, Heilongjiang Province, China,	[28]

5.	The human laryngeal cancer HEp-2 cell line	The autophagy was induced in HEp-2 cells along with CDDP-induced apoptosis, and inhibition of autophagy could result in potentiation of the proapoptotic effect of CDDP. Appropriate modulation of autophagy is necessary for sensitizing laryngeal cancer cells to CDDP treatment	Anti-Beclin 1 antibody (Santa Cruz, catalog number: sc-10086) recognizes a single 60-kd band on Western blot.	[29]
6.	Human laryngeal epidermoid carcinoma (HEp-2) cells	The BHAE triggered cell death by activation of apoptosis through ROS induction. Its ability to induce cell death through ROS generation leads to morphological and molecular modifications, cell cycle arrest and induction of apoptosis.	The Algerian Berberis hispanica alkaloids extract (BHAE)	[30]
7.	HEp-2 cell lines by *in vitro*	That AETE has immense potential to induce apoptosis by depolarizing the mitochondrial membrane. The in vivo results in TV and TW reduction following AETE administration clearly indicated the tumor regression potential of AETE against EAC-induced solid tumors.	Aqueous extract of Tagetes erecta (AETE)	[31]
8.	HEp-2 and KB cells	Genotoxic stress leads to down-regulation of Bcl-2 in HEp-2 and KB cells, which plays a decisive role in the outcome of stress in these cells.	Agents like carboplatin (Carb) and 5-fluorouracil (5-FU),	[32]
9.	HEp-2 cells	The possible mechanism of apoptosis as above was to decrease intracellular GSH, increase intracellular ROS and lose mitochondrial membrane potential.	Arsenic trioxide-As_2O_3	[33]
10.	HEp-2 cell lines.	Induction of Cdk inhibitor p21, inhibition of cyclin D1 and activation of caspase-3.	Indirubin-3-monooxime was purchased from Gibco Laboratories and dissolved in dimethyl sulfoxide (DMSO, Sigma).	[34]
11.	HEp-2 cells and African green monkey kidney (Vero) cells	Loss of membrane integrity	Combinatorial effects of geopropolis produced by *Melipona fasciculata* Smith	[35]
12.	The Human Laryngeal carcinoma (HEp-2)	Induces apoptosis in HEp-2 cells through by inducing oxidative damage and modulating apoptotic marker expressions. Inhibits cell proliferation in HEp-2 cells through ROS dependant mitochondrial mediated apoptosis as evidenced by elevation of ROS generation resulting in loss of DCM, oxidative DNA damage, and nuclear fragmentation. Further, modulates the apoptotic protein expression such as decreased Bcl-2, increased Bax leads to Cytochrome-c release from mitochondria, consequent activation of Caspase-9 and -3.	[6]-Shogaol	[36]
13.	HEp-2 (human laryngeal	Mitochondrial perturbation	Brazilian red propolis extract	[37]

	epidermoid carcinoma cell)			
14.	The laryngeal squamous carcinoma HEp-2 cell line	Down-regulated expression of MMP-2 and MMP-9 via ROS-mediated inhibition of phosphorylation in the ERK/MEK signaling pathway. Suppression of MMP-2 and MMP-9 protein expression by 9-HPbD-PDT may improve the therapy of laryngeal cancer by reducing metastasis.	9-HPbD, a novel chlorophyll derived photosensitizer, with a maximum absorption peak at 664 nm, was kindly provided by Kumho Life and Environmental Science Labo-ratory (Kwangju, Korea).	[38]
15.	Human epithelial type 2 (HEp-2) cells	Interfered its adhesion to the epithelial cells	Rhus coriaria were purchased from the local markets of Karaj city in 2016	[39]

The feruloiltyramine compound (Table 4) is also thought to be active as an anticancer. It was also reported that the extract of the Tinospora crispa plant originating from East Asia contains several compounds, one of which is feruloyltyramine, which can inhibit the proliferation of cancer cells MCF-7, HeLa cells, HEpG2 cells, Caov-3 cells, MDA-MB-231 cells, and 3T3 cells [40]

Santonin contained in the ethyl acetate extract of longan honey is a class of sesquiterpene compounds with biological activity as anti-therapeutic, anti-inflammatory, and fungicidal agents. Synthetic santonin derivatives such as spiro-isoxazolidine can inhibit PC-3 cells by inducing an 'arrest' process in the G1 cell cycle phase [41].

Table 6. Some of the chemical compounds or their derivatives found in this study as anticancer

No	Compound	Mechanism	Type Cancer cells	References
1.	3,4-dimethoxycinnamic acid	Effective components induced apoptosis by inhibiting tumor cell migration, activating caspase 3, and promoting ROS production.	The human breast cancer cells, MCF-7 (human breast cancer ER (+)) and MDA-MB-231 (human breast cancer ER (−)) cells, lung cancer A549 cells, and human colonic carcinoma HeLa cells were purchased from American Type Culture Collection (ATCC, USA).	[42]
2.	Cinnamic acid, 3-chlorocinnamic acid, 4-chlorocinnamic acid, and thionyl chloride	Strong inducers of apoptosis in A549 cells, both activating intrinsic caspase pathway and cell cycle arrest at the G0/ G1 phase. Ability to induce production of mitochondrial superoxide anions, inhibited cellular mobility.	Human colorectal adenocarcinoma (LoVo,ATCCÒ CCL-229TM), human ovary adenocarcinoma (SkOV-3, ATCCÒ HTB-77 TM), human lung non-small cell carcinoma (A549, ATCCÒ CCL-185TM), human mammary adenocarcinoma (MCF-7, ATCCÒ HTB-22 TM), human pancreatic adenocarcinoma (AsPC-1, ATCCÒ CRL-1682 TM) and human hepatocellular carcinoma (HepG-2, ATCCÒ HB-8065 TM) cell lines were generously gifted by Transgene SA (Illkirch, Strasbourg,	[43]

			France).	
3.	Esculetin	In vitro and in vivo antiproliferative activity in hepatocellular carcinoma, and its mechanisms involved initiating a mitochondrial-mediated, caspase-dependent apoptosis pathway.	The human ccRCC cell line 786-O was purchased from the National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China).	[44]
		in vitro and in vivo antiproliferative activity in hepatocellular carcinoma, and its mechanisms involved initiation of a mitochondrial-mediated, caspase-dependent apoptosis pathway.	The SMMC-7721 human hepatocellular carcinoma cell line was obtained from the Scientific Experiment Center of Liaoning Medical College (China)	[45]
		Increased the levels of Bax, cleaved caspase-3, cleaved-9 and cleaved poly (ADP-ribose) polymerase apoptosis-related proteins, and decreased the expression levels of the Bcl-2 anti-apoptotic protein.	A253 cells were cultured in modified RPMI 1640 medium (Welgene, Inc.)	[46]
		Binds to KEAP1 and inhibits its interaction with Nrf2 in pancreatic cancer cells.	PANC-1, MIA PaCa-2 and AsPC-1 cell lines	[20]
		Reduced the protein expression of CDK4 and cyclin D1, regulators of G1/S transition, in both cell lines	CMT-U27 and CF41.mg cell lines were purchased from ATCC (Manassas, VA, USA).	[47]
4.	N-trans-feruloyltyramine	Downregulation of COX-2 and iNOS via suppression of AP-1 and the JNK signalling pathway in RAW 264.7 macrophages.	RAW 264.7 cells and human embryonic kidney cells (HEK293) were purchased from the Korean Cell Line Bank (Seoul, Korea).	[48]
		Proliferation inhibition on HepG2 cells with IC50 value of 194 ± 0.894 µM.	Human hepatoma cells (HepG2 cells) and Human normal hepatocyte cells (L02 cells) were obtained from cell resource center of the Shanghai Academy of Sciences (Chinese Academy of Sciences, China),	[49]
5.	trans-N-feruloyltyramine (3) and trans-N-feruloyl-3-ethoxytyramine (4)	The alkaloids extract may probably induce extrinsic (TNFR1 mediated) as well as intrinsic (TP53 induced) apoptosis pathways in leukemic cell line.	Acute T cell leukemic (Jurkat E6-1, passage number 29) cell line was procured from National Centre of Cell Science (NCCS), Pune, India.	[50]
7.	6-mercaptopurine (6-MP)	In vitro cell cytotoxic and oxidative stress marker indices (lipid peroxide, superoxide dismutase, glutathione-SH, and protein carbonyl) were significantly reduced	Human neuroblastoma cell line (IMR32)	[51]
		Up-regulation of MRP4 and down-regulation of influx transporters played a major role in 6-MP resistance of CEM-MP5 cells.	The CCRF-CEM (American Type Culture Collection, Manassas, VA, CCL-119) cell line (hereafter referred as CEM) is a human T-lymphoblast cell line that was originally derived from a patient with acute lymphocytic leukemia.	[52]
		Inhibition ratios on biotin receptor overexpressed cell lines (MCF-7, HepG2) and lower cytotoxicity on normal cell lines (CHO). Compound 5 showed good activity, comparable to 6-MP against (MCF- 7, HepG2), and much lower cytotoxicity on (CHO).	Human breast cancer cell (MCF7), human hepatic carcinoma (HepG2) and biotin negative cell line, noncancerous Chinese hamster ovarian (CHO) cell lines.	[53]
8.	S-allylthio-6-mercaptopurine and its ribose derivate	boosted in vitro apoptotis in B-CLL cells from 10% to 38%, and decreased in vivo engraftment of B-CLL from 30% to 0.7%.	Human-mouse B-CLL model.	[54]

9.	γ- (9H-purine-6-yl). thiomethyl L-glutamate (6-MPG), a water-soluble derivative of 6-MP.	6-MPG against the secondary tumor is elicited by augmenting tumor specific T-cell production.	MethA growth in the BALB/C (nu/nu) mouse.	[55]
10.	4-(1,2,3-triazol-1-yl)coumarin	The antiproliferative role through arresting G2/M cell-cycle and inducing apoptosis	Human cancer cell lines, including human breast carcinoma MCF-7 cell, colon carcinoma SW480 cell and lung carcinoma A549 cell.	[56]
11.	2-phenylpyrimidine coumarin derivatives	Telomerase inhibitors	CNE2, KB, and Cal27 cell lines in vitro	[57]
12.	New thiazolylpyrazolyl coumarin derivatives	The mechanistic effectiveness in cell cycle progression, apoptotic induction and gene regulation, significant VEGFR-2 inhibition, stimulates the apoptotic death of MCF-7 cells, up regulated p53 gene expression and elevated Bax/Bcl-2 ratio which confirmed the mechanistic pathway, activation of caspases-7 and 9, effective apoptosis modulator and promising lead for future development of new anti-breast cancer agents.	Human cell lines, including breast MCF-7, lung A549, prostate PC3, liver HepG2 and normal melanocyte HFB4.	[58]
13.	Some new indole–coumarin hybrids	Flow cytometric cell cycle analysis of 4c exhibited apoptotic mode of cell death due to cell cycle arrest in the G2/M phase.	Human breast adenocarcinoma (MCF-7) cells	[59]
14.	Coumarin derivates	In this regard, many coumarin derivatives have been reported as intercalating agents, alkylating agents, topoisomerases inhibitors, hormone antagonists, angiogenesis inhibitors, antimitotic agents, apoptosis inducers, carbonic anhydrase inhibitors.	L1210, CEM Du 145, HeLa, MCF-7, A549, HeLa, SKNSH, HepG2 cells, COLO-205, THP-1 and HCT-116 cancer cell lines	[60]
		BRD4 inhibitor, arresting the cell cycle at the G0/G1 phase, induced MCF-7 cells apoptosis and reduced the expression of c-Myc protein, good in vivo and in vitro metabolic stability with a long half-life time	MCF-7, HepG2, WI-38 and HEK293T cells	[61]
15.	A series of hybrid molecules composed by a coumarin scaffold and a properly substituted trans-vinylbenzene moiety	Apoptosis-inducing activity	H460 lung carcinoma cells	[62]
16.	Coumarins	Carbonic anhydrase inhibitors, kinase inhibitors/Miscillaneous, antimitotic agents, aromatase and sulfatase inhibitors, angeogenesis inhibitors, cell cycle arrest causing agents, monocarboxylate transporters inhibitors, telomerase inhibitors, HSP 90 inhibitors.	Hep-G2, Panc-1, CCRF, SIH4, MDA-MB-468/MCF-7, SK-OV-3, CCR-CEM, HL-60, HOP-92, NCl-H460, HCT-116, SF-295, A549, CRL1548, CRL 1439, HeLa, SW620, A2780, Ketr-3, SGC-7901, MGC-803, Bcap-37, HEPG-2, SKBR3, Hep-2, HuCCA-1, MOLT-3, K562, Bel-7402,	[63]
17.	7-IP-based butynyl-amino derivatives	the MDR reverting ability,	LoVo colon cancer cell line	[64]
18.	trimeric triphenylethylene–coumarin hybrids	anti-tumor activities and binding affinity with DNA in intercalative mode	Hela, A549, K562 and MCF-7 cell lines were examined by the modified Mosmann's protocol	[65]
19.	2-amino-5-substitu ted-1,3,4-thiadiazole derivatives	Biological agents against EGFR kinase	MCF-7 and PC3 cell lines	[66]

20.	Quercetin 1,2-Dimethylhydrazine dihydrochloride (DMH, D161802) and Quercetin	Effectively reversed DMH-mediated oxidative stress and DNA damage through targeting NRF2/Keap1 signaling pathway.	Colorectal cancer.	[67]
21.	α-santonin derivatives	Inhibition on cell-cycle progression and leading to an increase of cells in G2/M, the DNA synthesis inhibition and cell cycle arrest, which probably lead to apoptosis activation.	HL-60 cell line	[68]
		Possessing the a-methylidene-g-butyrolactone group in their structure, displayed significant cytotoxic activities.	The cancer cell lines HL-60 (leukemia), SF-295 (central nervous system), HCT-8 (colon), MDA-MB-435 (melanoma), UACC-257 (melanoma), A549 (lung), OVACAR-8 (ovarian), A704 (renal), and PC3 (prostate)	[69]
		TNF-a protein as its preferable target	Tumor Necrosis Factor Alpha (TNF-a)	[70]
22.	Novel spiro-isoxazoline and spiro-isoxazolidine derivatives of α-santonin	showed concentration dependent inhibitory activity against NF-kB, p65	PC-3, THP-1 and MCF-7 cell lines	[71]
23.	α-santonin (a methylene-g butyrolactone)	These lead molecules were further studied for NF-kB, p65 transcription factor inhibitory activity which confirmed concentration dependent inhibition against NF-kB, p65	Human cancer cell lines (PC-3, HCT-15, A-549 and MCF-7).	[72]
24	α-santonin derived acetyl santonous acid 1,2,3-triazole derivatives	Reduced cytotoxicity and strong inhibition activity against the cell proliferation induced by the mitogens.	BALB/c mice T and B lymphocyte proliferation. 8.5	[73]

Another compound that is thought to play a role in inhibiting cancer is a coumarin derivative, namely 3(2,4-dichlorophenyl)-4-phenylcoumarin, which is present in Fraction 5 (Table 2). Phenyl coumarin group compounds have more potent cytotoxic activity than the cancer drug doxorubicin against colon cancer cells, with the lowest viability at a concentration of 10 (g/mL). Synthetic coumarin derivatives with anti-breast cancer potential, covering the articles published from 2015 to 2020 [74]. Thakur et al. [63], accomplished in vitro cytotoxic screening against HEp-2 cell line and illustrated SAR study of 4-methyl 2h-chromen-2-one derivatives. This compound is a derivative of Coumarin with IC_{50} value 9.798 ± 0.18 on hep2 cells [63]. A vigilant SAR (Figure 11) exploration verified that substitution of phenyl ring at position R with para-fluoro, ortho-OH and ortho-OCH_3 showed maximum activity (Table 7).

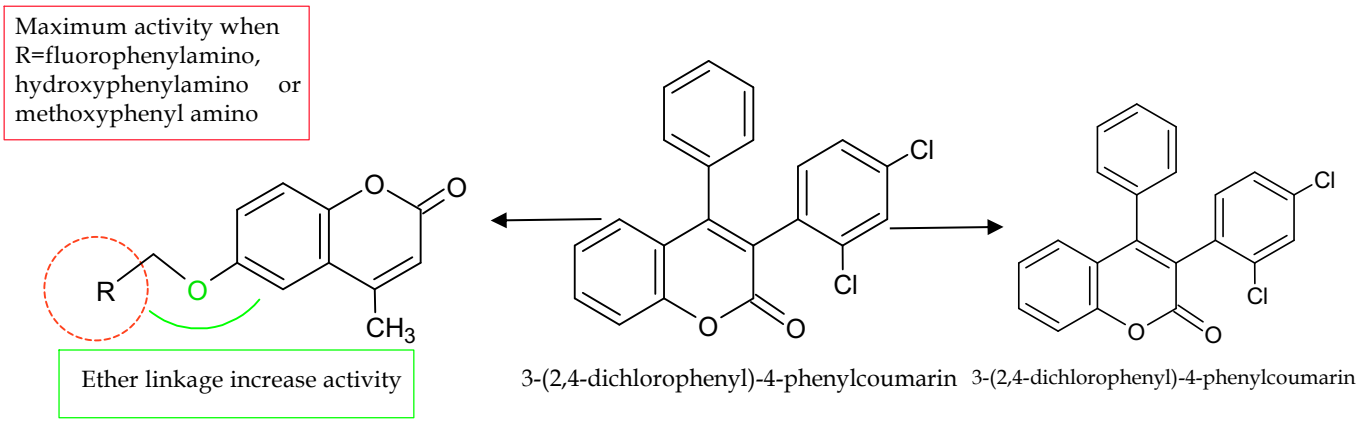
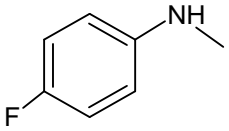
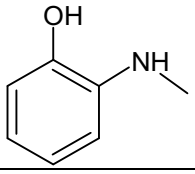
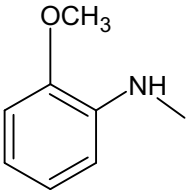


Figure 11. Comparison SAR study of 4-methyl-chromen-2-one derivatives [63] and another of coumarin derivative.

The ability of longan honey components and their derivatives has also been seen in various studies of several cell lines (Table 6). This provides information that the compounds that make up longan honey can inhibit HEp-2 cells through multiple mechanisms.

Table 7. In silico analysis of the substitution of the R position in 4-methyl-chromen-2-one [63]

No	R	IC_{50} HEp-2 cells (μM)
1		3.621 ± 0.10
2		4.196 ± 0.12
3		5.432 ± 0.15

3. CONCLUSION

Isolation and characterization of longan honey samples using FTIR and LC-MS/MS showed the presence of the following compounds: Xanthoxol glycosides, Santonin, Octadecanamide, Indole-3 carboxylaldehyde, 3,4-dimethoxycinnamic acid, Dimethyl esculetin, Tryptophan, O-acetyl-L- serine, D-glucose-6-phosphate, Feruloiltyramine, Lauryl diethanolamide, Taurine, 6-mercaptopurine, 3-(2,4-dichlorophenyl)-4-phenylcoumarin, 3',4'-dimethoxy-3-hydroxy-6-methylflavone and D-1-((3-carboxypropyl)amino)-1-deoxyfructose. The active compounds found in longan honey and suspected to be active against HEp-2 cells are thought to not only come from the flavonoid (quercetin) and phenolic groups (3,6-dimethoxycinnamic acid, phenyl coumarin, and dimethyl esculetin) but also from several compounds containing others such as Santonin, 6-mercaptopurine, and feruloyltyramine. Possible Mechanism of Honey to Inhibit HEp-2 Cells through several ways including: via caspase pathway, purine synthesis and other relevant mechanisms. However, this assumption needs to be tested further to obtain more precise information regarding the mechanism of inhibition of HEp-2 cells.

4. MATERIALS AND METHODS

4.1. Materials and Instruments

All chemicals and reagents in this study were used for analytical grade without any further purification. The sample used was longan honey from Central Java. The chemicals used are solvents for extraction (Methanol pa (FULLTIME), N-hexane pa (FULLTIME), Ethyl acetate pa (SmartLab), and Aquadest), TLC Silica gel 60 F254 (Merck), Silica gel 60 F254 (Merck), Na_2SO_4 anhydrous pa (Merck).

The equipment used includes glassware, separating funnel, magnetic stirrer, Buchner funnel, rotary evaporator, vacuum pump, Whatman filter paper, microplate reader, biosafety Cabinet Level-2 (Nuaire, USA), CO_2 incubator (Binder, Germany), centrifuge (Tommy, Japan), inverted microscope (Nikon, Japan), T25 flask (Corning, USA), hemocytometer device (Improved Neubauer), 96-wells Tissue Culture Plate (Corning, USA), 12-wells Tissue Culture Plate (Corning, USA), pipette aids, volumetric pipettes, media bottles, FTIR (Bruker Inc.), and LC-MS/MS (Waters, USA).

4.1.1 Instrumentation

Table 8. Conditions and Details Instrumentation

Conditions	Details
Chromatographic Separation:	
C System	Ultra Performance Liquid Chromatogphy (UPLC)
Column	C18 (1.8 µm 2.1x50 mm) BEH
Temperature	50°C (Column), 25°C (room)
Mobile phase	Water + 0.1%Formic acid (A) and Acetonitril + 0.1% Formic acid (B)
Flow rate	0.2 mL/min (step gradien) running 23 minute
Injection Volume	5 µL (filter through 0.2 µm syring filter first)
Mass Spectrometry:	ES (electrospray ionization)
System	
Mode	Positive mode
Mass analysis range	50 – 1500 m/z
Source Temperature	100°C
Desolvation Temperature	350°C
Cone gas flow	0 L/hr
Desolvation gas flow	793 L/hr
Collision energi	4 Volt
Rampt Colision energi	25-70 volt

Table 9. LC-MS Analysis Detail

Instrument	Specification	Details
LC System	ACQUITY UPLC*H-Class System (waters, USA)	UPLC (Ultra Performance Liquid Chromatography)
LC Column	ACQUITY UPLC*BEH C18 (1.8 µm 2.1x50 mm) (waters, USA)	UPLC Column BEH (Ethylene Bridge Hybride)
Mass Spectrometer	Xevo G2-S Qtof (waters, USA)	Quadrupole time-of-flight mass spectrometry

4.2. Procedure

4.2.1. Honey Preparation

Longan honey was extracted and continued by liquid-liquid partitioning to obtain an extract so that it was obtained water extract (polar), ethyl acetate (semi-polar), and n-hexane (nonpolar) longan honey. The honey extract was separated by TLC and continued by fractionation by a gravity chromatography column. Each fraction was retested for its inhibitory activity and characterized by LC-MS/MS and FTIR, including honey extract, which provided the most significant inhibition to determine the suspected compounds that played a role in inhibiting the growth of HEp-2 cells.

4.2.2. Honey Extraction

A total of 100 g of honey samples were added with 300 mL of methanol and stirred using a magnetic stirrer for 30 minutes. Then let it sit in a fume hood for 24 hours. Using filter paper, the honey extract is then separated from the residue that settles with the filtrate. The honey methanol extract filtrate was then concentrated using a rotary evaporator at a temperature of 64°C to obtain a concentrated honey methanol extract.

4.2.3. Liquid-Liquid Partition

The concentrated honey methanol extract was separated by the liquid-liquid partition method successively using n-hexane and ethyl acetate as solvents. The concentrated extract of honey methanol was dissolved in 200 mL of water-methanol (3:7), then put into a separating funnel and added with 100 mL of n-hexane as solvent. The mixture is then shaken for 5 minutes and allowed to stand until it is completely separated into two parts. The N-hexane fraction above was divided, while the water-methanol below was re-partitioned with N-hexane until the n-hexane fraction was clear and the n-hexane honey fraction was obtained. Furthermore, the water-methanol fraction of honey was added with 100 mL of ethyl acetate solvent and partitioned using the same method with n-hexane solvent to get a precise colored ethyl acetate fraction. The water-methanol, n-hexane, and honey ethyl acetate fractions were added with 5 g of anhydrous Na_2SO_4 to remove the remaining water content and filtered using a Buchner funnel to separate the Na_2SO_4. All honey

fractions were concentrated using a rotary evaporator, n-hexane fraction at 48°C, ethyl acetate fraction at 54°C, and water-methanol fraction at 64°C.

4.2.4. Thin-Layer Chromatography

Separation by Thin Layer Chromatography (TLC) was carried out using a silica gel 60 F254 TLC plate and a mixture of several eluents consisting of n-hexane: ethyl acetate: acetone with a composition of 1:3:1. The spots on the TLC plate were monitored under UV lamps at 254 nm and UV 365 nm.

4.2.5. Gravity Column Chromatography Fractionation

The solvent composition that produces the best TLC separation and the best spot separation stain is then used as the mobile phase in gravity column chromatography. Silica gel 60 F254 was suspended first with n-hexane and put into a column whose bottom was plugged with cotton using a dropper until the silica gel height reached 3/4 of the column height. Then the n-hexane is removed and collected again. The extract was dissolved with a small amount of acetone solvent and added with silica gel as much as the amount of section in a ratio of 1:1. Then inserted into the column and eluted using the gradient method. The results of the column chromatography obtained were collected in vials of 50 mL and 100 mL of each fraction. All fractions separated by column chromatography were then analyzed using thin layer chromatography with n-hexane: ethyl acetate: acetone (1:3:1) as eluent and observed under UV lamps at 254 nm and 366 nm to see stains with the same Rf. Fractions with the same stain on thin layer chromatography are combined and the solvent is evaporated.

4.2.6. Analysis FTIR

A total of 1 mg of the active honey fraction was crushed with 100 mg of KBr homogeneously. The infrared absorption was measured at a wave number of 4000 - 450 cm^{-1}, and the functional groups were analyzed by comparing the wave numbers in the sample with a reference.

4.2.7. Analysis LC-MS/MS

As much as 1 mg of the active honey fraction was weighed and dissolved in methanol. 10 L of the sample was taken and injected into LC-MS/MS through column C-18 (2 x 150 mm) with a flow rate of 0.3 mL/min. The chromatogram and mass spectrophotometer analysis were then analyzed using the MassLynx software (Version 4.1). Identifying the

structure of the chemical compounds detected in the LC-MS/MS, was done by comparing the base peak samples with the database on the Massbank website. The column specification or stationary phase used in LC-MS/MS is the ACQUITY UPLC®BEH C18 column. This column has a reverse phase because the stationary phase is nonpolar while the mobile phase is polar. The mobile phase is a mixture of methanol-water and acetonitrile-water solvents. However, the best separation occurs when using an acetonitrile-water solvent.

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REFERENCES

[1] Pasini F, Gardini S, Marcazzan GL, Caboni MF. Buckwheat honeys: Screening of composition and properties. Food Chem. 2013; 141: 2802–2811. https://doi.org/10.1016/j.foodchem.2013.05.102

[2] Spilioti E., Jaakkola M, Tolonen T, Lipponen M, Virtanen V, Chinou I, Kassi E, Karabournioti S, Moutsatsou P. Phenolic Acid Composition, Antiatherogenic, and Anticancer Potential of Honeys Derived from Various Regions in Greece. PLOS ONE. 2014; 9(4): 1–10. https://doi.org/10.1371/journal.pone.0094860

[3] Fathoni A, Sumarlin L, Fuady Hanief F, Sukandar D. Isolation And Cytotoxic Activity Of The β-Carotene Combination Of Trigona Honey And Namnam Leaves Extract (Cynometra cauliflora). Elkawnie: Journal of Islamic Science and Technology. 2021; 7(1): 52-66. http://dx.doi.org/10.22373/ekw.v7i1.8696

[4] Wasagu RSU, Shehu S, Mode YD. Comparative Proximate Composition And Antioxidant Vitamins Contents of Two Honey Varieties (Light Amber And Dark Amber) From Sokoto State, Nigeria. Bayero J. Pure Appl. Sci. 2013; 6(2): 118 – 120. http://dx.doi.org/10.4314/bajopas.v6i2.25

[5] Fathoni A., Sumarlin L, Putri JR, Fitriana N. Antioxidant Activity Of Mixed Katuk LeafExtract And Honey. EduChemia. 2020; 5(2): 168-179. http://dx.doi.org/10.30870/educhemia.v5i2.7275

[6] Hossen MS, Ali MY, Jahurul MHA, Abdel-Daim MM, Gan SH, Khalil MI. Beneficial roles of honey polyphenols against some human degenerative diseases: A review. Pharm. Rep. 2017; 69: 1194–1205. https://doi.org/10.1016/j.pharep.2017.07.002

[7] Almasaudi S. Review: The antibacterial activities of honey. Saudi J. Biol. Sci. 2021; 28: 2188-2196. https://doi.org/10.1016/j.sjbs.2020.10.017

[8] Gül A, Pehlivan T. Antioxidant activities of some monofloral honey types produced across Turkey. Saudi J. Biol. Sci. 2018; 25(6): 1056-1065. https://doi.org/10.1016/j.sjbs.2018.02.011

[9] Bouali, N, Hamadou WS, Badraoui R, Lajimi RH, Hamdi A, Alreshidi M, Adnan M, Soua Z, Siddiqui AJ, Noumi E, Snoussi M. Phytochemical Composition, Antioxidant, and Anticancer Activities of Sidr Honey: In Vitro and In Silico Computational Investigation. Life. 2023; 13: 35. https://doi.org/10.3390/life13010035

[10] Sumarlin LO, Muawanah A, Afandi FR, Adawiah A. Inhibitory Activity of HEp-2 Cells by Honey from Indonesia. Jurnal Kimia Sains dan Aplikasi. 2019; 22(6): 317-325. https://doi.org/10.14710/jksa.22.6.317-325

[11] Alghamdi BA, Alshumrani ES, Bin Saeed MS, Rawas GM, Alharthi NT, Baeshen MN, Helmi NM, Alam MZ, Suhail M. Analysis of sugar composition and pesticides using HPLC and GC–MS techniques in honey samples collected from Saudi Arabian markets. Saudi J. Biol. Sci**.** 2020; 27(12): 3720-3726. https://doi.org/10.1016/j.sjbs.2020.08.018

[12] Ng L, Wu, S. Antiproliferative Activity of Cinnamomum cassia Constituents and Effects of Pifithrin-Alpha on Their Apoptotic Signaling Pathways in HEp G2 Cells. Evid Based Complement Alternat Med. 2011;. 9(2): 1–6. https://doi.org/10.1093/ecam/nep220

[13] Siswarni MZ, Putri YI, Rinda PR. Ekstraksi Kuersetin Dari Kulit Terong Belanda (Solanum betaceum Cav.) Menggunakan Pelarut Etanol Deangan Metode Maserasi dan Sokletasi. Jurnal Teknik Kima USU. 2017; 6(1): 36–42. https://doi.org/10.32734/jtk.v6i1.1563

[14] Mahasuari NPS, Paramita NLP, Putra AAGRY. Effect Of Methanol Concentratıon As A Solvent On Total Phenolıc And Flavonoıd Content Of Beluntas Leaf Extract (Pulchea İndica L.). J. Pharm. Sci. Appl**.** 2020; 2(2): 77-88. https://doi.org/10.24843/JPSA.2020.v02.i02.p05

[15] Horizon, Pujiastuti B, Kurnia D, Sumiarsa D, Supratman U, Shiono Y. Kuersetin dan Kuersetin-3- O -Glukosida dari Kulit Batang Sonneratia Alba (Lythraceae). Jurnal Kimia VALENSI. 2015; 1(1): 33–38. https://doi.org/10.15408/jkv.v0i0.3151

[16] Hashemzaei M, Far AD, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, Sadegh SE, Tsarouhas K, Kouretas D, Tzanakakis G, Nikitovic D, Anisimov NY, Spandidos DA, Tsatsakis AM, Rezaee R. Anticancer and apoptosis - inducing effects of quercetin in vitro and in vivo. Oncol. Rep. 2017; 38(2): 819–828. https://doi.org/10.3892/or.2017.5766

[17] Wang G, Zhang J, Liu L, Sharma S, Dong Q. Quercetin Potentiates Doxorubicin Mediated Antitumor Effects against Liver Cancer through p53/Bcl-xl. PLOS ONE. 2012; 7(12): 1–12. https://doi.org/10.1371/journal.pone.0051764

[18] Ahmed S, Othman NH. Honey as a potential natural anticancer agent: A review of its mechanisms. J Evid Based Complementary Altern. Med. 2013; 2013: 829070. https://doi.org/10.1155/2013/829070

[19] Abubakar MB, Abdullah WZ, Sulaiman SA, Suen AB. A Review of Molecular Mechanisms of the Antileukemic Effects of Phenolic Compounds in Honey. Int J Mol. Sci. 2012; 13(11):15054–15073. https://doi.org/10.3390/ijms131115054

[20] Arora R, Sawney S, Saini V, Steffi C, Tiwari M, Saluja D. Esculetin induces antiproliferative and apoptotic response in pancreatic cancer cells by directly binding to KEAP1. Mol Cancer. 2016; 64: 1–15. https://doi.org/10.1186/s12943-016-0550-2

[21] Wu D, Chen L, Teh J, Sim E, Schlundt J, Conway PL. Honeys with anti-inflammatory capacity can alter the elderly gut microbiota in an ex vivo gut model. Food Chem. 2022; 392: 133229. https://doi.org/10.1016/j.foodchem.2022.133229

[22] Devi JR, Thangam EB. Mechanisms of Anticancer Activity of Sulforaphane from Brassica oleracea in HEp-2 Human Epithelial Carcinoma Cell Line. Asian Pac J Cancer Prev. 2012; 13(5): 2095–2100. https://doi.org/10.7314/APJCP.2012.13.5.2095

[23] Kahlin AZ, Helander S, Wennerstrand P, Vikingsson S, Mårtensson L-G, Appell ML. Pharmacogenetic studies of thiopurine methyltransferase genotype-phenotype concordance and effect of methotrexate on thiopurine metabolism. Basic Clin Pharmacol Toxicol. 2021; 128: 52– 65. https://doi.org/10.1111/bcpt.13483

[24] Yang Y, Zhou S, Ouyang R, Yang Y, Tao H, Feng K, Zhang X, Xiong F, Guo N, Zong T, Cao P, Li Y, Miao Y. Improvement in the Anticancer Activity of 6-Mercaptopurine via Combination with Bismuth(III). Chem. Pharm. Bull., 2016; 64: 1539-1545. https://doi.org/10.1248/cpb.c15-00949

[25] Branco CDS, Émilin Dreher de Lima EDD, Rodrigues TS, Scheffel TB, Scola G, Laurino CCFC, Moura S, Salvador M. Mitochondria and redox homoeostasis as chemotherapeutic targets of Araucaria angustifolia (Bert.) O. Kuntze in human larynx HEp-2 cancer cells. Chem. Biol. Interact., 2015; 231:108–118. https://doi.org/10.1016/j.cbi.2015.03.005

[26] Shao F, Lv M, Zheng Y, Jiang J, Wang Y, Lv L, Wang J. The anti-tumour activity of rLj-RGD4, an RGD toxin protein from Lampetra japonica, on human laryngeal squamous carcinoma Hep-2 cells in nude mice. Biochimie., 2015; 119: 183-191. https://doi.org/10.1016/j.biochi.2015.11.004

[27] Zhang F, Yang B, Shi S, Jiang X. RNA interference (RNAi) mediated stable knockdown of protein caseinkinase 2-alpha (CK2α) inhibits migration and invasion and enhancescisplatin-induced apoptosis in HEp-2 laryngeal carcinoma cells. Acta Histochem., 2014; 116: 1000–1006. https://doi.org/10.1016/j.acthis.2014.04.001

[28] Miao S, Mao X, Pei R, Miao S, Xiang C, Lv Y, Yang X, Sun J, Jia S, Liu Y. Lepista sordida polysaccharide induces apoptosis of Hep-2 cancer cells via mitochondrial pathway. Int. J. Biol. Macromol., 2013; 61: 97– 101. https://doi.org/10.1016/j.ijbiomac.2013.06.052

[29] Kang R, Wang ZH, Wang BQ, Zhang CM, Gao W, Feng Y, Bai T, Zhang HL, Pu HH, Wen SX. Inhibition of autophagy-potentiated chemosensitivity to cisplatin in laryngeal cancer Hep-2 cells. Am J Otolaryngol., 2012; 33: 678–684. https://doi.org/10.1016/j.amjoto.2012.05.005

[30] Boudjlida A, Kaci S, Karaki S, Benayad T, Rocchi P, Smati D, Aouichat SB. Berberis hispanica alkaloids extract induced cell death and apoptosis in human laryngeal cancer cells Hep-2. S. Afr. J. Bot., 2019; 125: 134–141. https://doi.org/10.1016/j.sajb.2019.04.006

[31] Barhoi D, Upadhaya P, Barbhuiya SN, Giri A, Giri S. Extracts of Tagetes erecta exhibit potential cytotoxic and antitumor activity that could be employed as a promising therapeutic agent against cancer: A study involving in vitro and in vivo approach. Phytomedicine Plus, 2022; 2: 100187. https://doi.org/10.1016/j.phyplu.2021.100187

[32] Singh S, Chhipa RR, Vijayakumar MV, Bhat MK. DNA damaging drugs-induced down-regulation of Bcl-2 is essential for induction of apoptosis in high-risk HPV-positive HEp-2 and KB cells. Cancer Lett., 2006; 236: 213–221. https://doi.org/10.1016/j.canlet.2005.05.024

[33] Cheng B, Yang X, An L, Gao B, Liu X. Arsenic trioxide-induced apoptosis of Hep-2 cell line through modulating intracellular glutathione (GSH) level. Auris Nasus Larynx. 2010; 37: 89–94. https://doi.org/10.1016/j.anl.2009.04.016

[34] Kameswaran TR, Ramanibai R. Indirubin-3-monooxime induced cell cycle arrest and apoptosis in Hep-2 human laryngeal carcinoma cells. Biomed. Pharmacother., 2009; 63: 146-54. https://doi.org/10.1016/j.biopha.2008.03.005

[35] Bartolomeua AR, Herreraa YF, da Silvaa LM, Romagnolia GG, de Oliveirab DE, Sforcina JM. Combinatorial effects of geopropolis produced by Melipona fasciculata Smith with anticancer drugs against human laryngeal epidermoid carcinoma (HEp-2) cells. Biomed. Pharmacother., 2016; 81: 48–55. https://doi.org/10.1016/j.biopha.2016.03.049

[36] Annamalaia G, Kathiresana S, Kannappanb N. [6]-Shogaol, a dietary phenolic compound, induces oxidative stress mediated mitochondrial dependant apoptosis through activation of proapoptotic factors in Hep-2 cells. Biomed. Pharmacother., 2016; 82: 226–236. https://doi.org/10.1016/j.biopha.2016.04.044

[37] da Silva Frozzaa CO, Santosa DA, Rufattob LC, Minettob L, Scariot FJ, Echeverrigaray S, Piche CT, Mourab S, Padilhaf FF, Borsukf S, Savegnago L, Collares T, Seixas FK, Dellagostin O, Elya MR, Henriquesa JAP. Antitumor activity of Brazilian red propolis fractions against Hep-2 cancer cell line. Biomed. Pharmacother., 2017; 91: 951–963. https://doi.org/10.1016/j.biopha.2017.05.027

[38] Huankang Z, Bo S, Swinarska JT, Wen L, Kuanlin X, Peijie H. 9- Hydroxypheophorbide α-mediatedphotodynamic therapy induces matrixmetalloproteinase-2 (MMP-2) and MMP-9 down-regulation in Hep-2 cells viaROS-mediated suppression of the ERK pathway. Photodiagnosis Photodyn Ther, 2014; 11: 55—62. https://doi.org/10.1016/j.pdpdt.2013.12.001

[39] Khodaii Z, Eslami , Kamalinejad M, Mirzaei A, Natanzia MM. Evaluation of aqueous-extracts from four aromatic plants for their activity against Candida albicans adhesion to human HEp-2 epithelial cells. Gene RepMM.orts, 2020; 18:100554. https://doi.org/10.1016/j.genrep.2019.100554

[40] Rahman MM, Khan MA. Anticancer Potential of South Asian Plants. Nat. Prod. Bioprospect. 2013; 3(3):74–88. https://doi.org/10.1007/s13659-013-0027-6

[41] Khazir J, Singh PP, Reddy DM, Hyder I, Shafi S, Sawant SD, Chashoo G, Mahajan A, Alam MS, Saxena AK, Arvinda S, Gupta BD, Kumar HM. Synthesis and anticancer activity of novel spiro-isoxazoline and spiro- isoxazolidine derivatives of α-santonin. Eur J Med Chemy. 2013; 63: 279–289. https://doi.org/10.1016/j.ejmech.2013.01.003

[42] Xuan H, Wang Y, Li A, Fu C, Wang Y, Peng W. Bioactive Components of Chinese Propolis Water Extract on Antitumor Activity and Quality Control. Evid. Based Complementary Altern. Med. 2016; 9. https://doi.org/10.1155/2016/9641965

[43] Assaleh MH, Bjelogrlic SK, Prlainovic N, Cvijetic I, Bozic A, Arandjelovic I, Vukovic D, Marinkovic A. Antimycobacterial and anticancer activity of newly designed cinnamic acid hydrazides with favorable toxicity profile. Arab. J. Chem., 2022; 15(1): 103532. https://doi.org/10.1016/j.arabjc.2021.103532

[44] Duan J, Shia J, Ma X, Xuan Y, Li P, Wang H, Fan Y, Gong H, Wang L, Pang Y, Pang S, Yan Y. Esculetin inhibits proliferation, migration, and invasion of clear cell renal cell carcinoma cells. Biomed. Pharmacother., 2020; 125: 110031. https://doi.org/10.1016/j.biopha.2020.110031

[45] Wang J, Lu ML, Dai HL, Zhang SP, Wang HX, Wei N. Esculetin, a coumarin derivative, exerts in vitro and in vivo antiproliferative activity against hepatocellular carcinoma by initiating a mitochondrial-dependent apoptosis pathway. Braz J Med Biol Res, 2015; 48(3): 245-253. https://doi.org/10.1590/1414-431X20144074

[46] Park SB, Jung WK, Kim HR, Yu HY, Kim YH, Kim J. Esculetin Has Therapeutic Potential Via The Proapoptotic Signaling Pathway In A253 Human Submandibular Salivary Gland Tumor Cells. Exp. Ther. Med., 2022; 24(2): 533. https://doi.org/10.3892/etm.2022.11460

[47] Choi J, Yoo MJ, Park SY, Seol JW. Antitumor Effects of Esculetin, a Natural Coumarin Derivative, against Canine Mammary Gland Tumor Cells by Inducing Cell Cycle Arrest and Apoptosis. Vet. Sci., 2023; 10: 84. https://doi.org/10.3390/vetsci10020084

[48] Jiang Y, Yu L, Wang MH. N-trans-feruloyltyramine inhibits LPS-induced NO and PGE2 production in RAW 264.7 macrophages: Involvement of AP-1 and MAP kinase signalling pathways. Chem. Biol. Interact., 2015; 235: 56–62. https://doi.org/10.1016/j.cbi.2015.03.029

[49] Gao X, Cong Wang C, Zhongqin Chena, Yue Chena, Ramesh Kumar Santhanama, Zihan Xuea, Qiqi Maa, Qingwen Guoa, Wei Liua, Min Zhangb, Haixia Chena Effects of N-trans-feruloyltyramine isolated from laba garlic on antioxidant, cytotoxic activities and H_2O_2-induced oxidative damage in HepG2 and L02 cells. Food Chem. Toxicol, 130 (2019) 130–141. https://doi.org/10.1016/j.fct.2019.05.021

[50] Basaiyye SS, Naoghare PK, Kanojiya S, Bafana A, Arrigo P, Kannan Krishnamurthi K, Saravanadevi Sivanesan S. Molecular mechanism of apoptosis induction in Jurkat E6-1 cells by Tribulus terrestris alkaloids extract. J. Tradit. Complement. Med., 2018; 8(3): 410-419. https://doi.org/10.1016/j.jtcme.2017.08.014

[51] Kevadiya BD, Chettiar SS, Rajkumar S, Bajaj HC, Gosai KA, Brahmbhatt H. Evaluation of clay/poly (L-lactide) microcomposites as anticancer drug, 6-mercaptopurine reservoir through in vitro cytotoxicity, oxidative stress markers and in vivo pharmacokinetics. Colloids Surf B Biointerfaces., 2013; 112: 400–407. https://doi.org/10.1016/j.colsurfb.2013.07.008

[52] Peng XX, Shi Z, Damaraju VL, Huanga XC, Kruhd GD, Wu HC, Zhoua Y, Tiwari A, Fub L, Cass CE, Chena ZS. Up-regulation of MRP4 and down-regulation of influx transporters in human leukemic cells with acquired resistance to 6-mercaptopurine. Leuk. Res., 2008; 32(5): 799–809. https://doi.org/10.1016/j.leukres.2007.09.015

[53] Talib, AB, Mohammed MH. Preparation, characterization and preliminary cytotoxic evaluation of 6-mercaptopurine-coated biotinylated carbon dots nanoparticles as a drug delivery system. Materials Today: Proceedings. 2023; 80(3): 2327-2333. https://doi.org/10.1016/j.matpr.2021.06.341

[54] Miron T, Wilchek M, Shvidel L, Berrebi A, Arditti FD. S-allyl derivatives of 6-mercaptopurine are highly potent drugs against human B-CLL through synergism between 6-mercaptopurine and allicin. Leuk. Res., 2012; 36(12): 1536–1540. https://doi.org/10.1016/j.leukres.2012.08.023

[55] Kashida T, Narasaki N, Sakai A, Tsujihara K, Tsuzurahara K, Naito K, Takeyama S. Study on the mechanism of immunopotentiating antitumor effect of 6-MPG, a water-soluble derivative of 6-mercaptopurine. Immunopharmaco., 1997; 37(1): 95–104. https://doi.org/10.1016/S0162-3109(97)00039-8

[56] Zhang W, Li Z, Zhou M, Wu F, Hou X, Luo H, Liu H, Han X, Yan G, Ding Z, Li R. Synthesis and biological evaluation of 4-(1,2,3-triazol-1-yl) coumarin derivatives as potential antitumor agents. Bioorganic Med. Chem. Lett., 2014; 24(3): 799–807. https://doi.org/10.1016/j.bmcl.2013.12.095

[57] Lv N, Sun M, Liu C, Li J. Design and synthesis of 2-phenylpyrimidine coumarin derivatives as anticancer agents. Bioorganic Med. Chem. Lett., 2017; 27(19): 4578–4581. https://doi.org/10.1016/j.bmcl.2017.08.044

[58] Mohamed TK, Batran RZ, Elseginy SA, Ali MM, Abeer E. Mahmoudc AE. Synthesis, anticancer effect and molecular modeling of new thiazolylpyrazolyl coumarin derivatives targeting VEGFR-2 kinase and inducing cell cycle arrest and apoptosis. Bioorg. Chem., 2019; 85: 253–273. https://doi.org/10.1016/j.bioorg.2018.12.040

[59] Kamath PR, Sunil D, Ajees AA, Pai KSR, Das S. Some new indole–coumarin hybrids; Synthesis, anticancer and Bcl-2 docking studies. Bioorg. Chem., 2015; 63: 101–109. https://doi.org/10.1016/j.bioorg.2015.10.001

[60] Al-Warhi T, Sabt A, Elkaeed EB, Eldehna WM. Recent advancements of coumarin-based anticancer agents: An up-to-date review. Bioorg. Chem., 2020; 103: 104163. https://doi.org/10.1016/j.bioorg.2020.104163

[61] Cui QH, Li WB, Wang ZY, Xu KY, Wang S, Shi JT, Zhang LW, Chen SW. Design, synthesis and biological evaluation of coumarin derivatives as potential BRD4 inhibitors. Bioorg. Chem., 2022; 128: 106117. https://doi.org/10.1016/j.bioorg.2022.106117

[62] Belluti F, Fontana G, Bo LD, Carenini N, Giommarelli C, Zunino F. Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: Identification of novel proapoptotic agents. Bioorg. Med. Chem., 2010; 18(10): 3543–3550. https://doi.org/10.1016/j.bmc.2010.03.069

[63] Thakur A, Singla R, Jaitak F. Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies. Eur. J. Med. Chem., 2015; 101: 476-495. https://doi.org/10.1016/j.ejmech.2015.07.010

[64] Bisi A, Cappadone C, Rampa A, Farruggia G, Sargenti A, Belluti F, Di Martino RMC, Malucelli E, Meluzzi A, Iotti S, Gobbi S. Coumarin derivatives as potential antitumor agents: Growth inhibition, apoptosis induction and multidrug resistance reverting activity. Eur. J. Med. Chem., 2017; 127:577-585. https://doi.org/10.1016/j.ejmech.2017.01.020

[65] Zhang L, Yao YC, Gao MY, Rong RX, Wang KR, Xiao-Liu Li XL, Chen H. Anticancer activity and DNA binding property of the trimers of triphenylethylene–coumarin hybrids. Chin Chem Lett., 2016; 27(11): 1708–1716. https://doi.org/10.1016/j.cclet.2016.05.027

[66] Natarajan R, Pasumalai M, Bhandare RR, Subramani AK, Shaik AB. 2D-Quantitative structure activity relationship (QSAR) modeling, docking studies, synthesis andin-vitro evaluation of 1,3,4-thiadiazole tethered coumarin derivatives as antiproliferative agents. J. Saudi Chem. Soc., 2021; 25(7): 101279. https://doi.org/10.1016/j.jscs.2021.101279

[67] Darband SG, Sadighparvar S, Yousefic B, Kaviani M, Pakdel FG, Mihanfar A, Rahimi Y, Mobaraki, Majidinia M. Quercetin attenuated oxidative DNA damage through NRF2 signaling pathway in rats with DMH induced colon carcinogenesis, Life Sci., 2020; 253: 117584. https://doi.org/10.1016/j.lfs.2020.117584

[68] Ferreira JRO, Cavalcanti BC, da Costa PM, de Arantes FFP, de Alvarenga ES, Maltha RGA, de Almeida Barbosa LC, Militão GCG, Pessoa C, Paulo Michel Pinheiro Ferreira PMP. Induction of G2/M arrest, caspase activation and apoptosis by a-santonin derivatives in HL-60 cells. Toxicol In Vitro., 2013; 27(5): 1458–1466. https://doi.org/10.1016/j.tiv.2013.03.010

[69] Arantes FFP, Barbosa LCA, Alvarenga ES, Demuner AJ, Bezerra DP, Ferreira JRO, Letı´cia V. Lotufo C, Pessoa C, Moraes MO. Synthesis and cytotoxic activity of α-santonin derivatives. Eur. J. Med. Chem., 2009; 44(9): 3739–3745. https://doi.org/10.1016/j.ejmech.2009.03.036

[70] Chinthakindi PK, Singh J, Gupta S, Nargotra A, Mahajan P, Kaul A, Ahmed Z, Koul S, Sangwan PL. Synthesis of α-santonin derivatives for diminutive effect on T and B-cell proliferation and their structure activity relationships. Eur. J. Med. Chem., 2017; 127: 1047-1058. https://doi.org/10.1016/j.ejmech.2016.11.018

[71] Khazir J, Singh PP, Reddy DM, Hyder I, Shafi S, Sawant SD, Chashoo G, Mahajan A, Alam MS, Saxena AK, Arvinda S, Gupta BD, Kumar HMS. Synthesis and anticancer activity of novel spiro-isoxazoline and spiro-isoxazolidine derivatives of α-santonin. Eur. J. Med. Chem., 2013; 63: 279-289. https://doi.org/10.1016/j.ejmech.2013.01.003

[72] Khazir J, Riley DL, Chashoo G, Mir BA, Liles D, Islam MA, Singh SK, Vishwakarma RA, Pilcher LA. Design, synthesis and anticancer activity of Michael-type thiol adducts of α-santonin analogue with exocyclic methylene. Eur. J. Med. Chem., 2015; 101: 769-779. https://doi.org/10.1016/j.ejmech.2015.07.022

[73] Dangroo NA, Singh J, Dar AA, Gupta N, Chinthakindi PK, Kaul A, Khuroo MA, Sangwan PL. Synthesis of α-santonin derived acetyl santonous acid triazole derivatives and their bioevaluation for T and B-cell proliferation. Eur. J. Med. Chem., 2016; 120: 160-169. https://doi.org/10.1016/j.ejmech.2016.05.018

[74] Huo FSX, Guo Z. Anti-breast Cancer Potential of Natural and Synthetic Coumarin Derivatives. Curr Top Med Chem. 2021; 21(18): 1692-1709. http://dx.doi.org/10.2174/1568026621666210303145430