International Journal of Agro Nutrifood Practices
Vol-3, Issue-4, December 2023
e-ISSN: 2583-066X

Anticancerous Properties of *Tetragonula travancorica* (stingless bee) Honey on Reproductive Cancer Cells

Namboorimadathil M. Nahala[1*], Chennattu M. Pareeth[2], K. P. Safna Hussan[3], Thekkekara D Babu[4], K. B. Soni[5], V. S. Amritha[6*]

[1, 5] Department of Agricultural Biotechnology, College of Agriculture, Thiruvananthapuram, Kerala, India
[2, 3, 4] Department of Biochemistry, Amala Cancer Research Centre, Thrissur, Kerala, India
[6] Department of Entomology, College of Agriculture, Thiruvananthapuram, Kerala, India
*Corresponding Author's Email: [1] nahala7nm@gmail.com, [6] amritvs@gmail.com

Abstract

Honey from stingless bee is highly regarded as a medicine in many global communities due to its nutritional and therapeutic value. There is a comparatively restricted amount of research on the biological characteristics of stingless bee honey (SBH) in contrast to Indian bee honey (IBH). Therefore, the objective of this research is to explore the anti-cancer properties of Tetragonula travancorica honey. In the present investigation, antioxidant activity of honey was evaluated using ABTS, DPPH and SOD radical scavenging assays. Anti-proliferative effects was examined on various cell lines using the MTT assay. Liquid chromatography-mass spectrometry (LCMS) analysis was employed to characterize the honey. Binding capabilities of compounds identified in SBH on estrogen receptors (ER) α and ER β were explored through Schrödinger Maestro software. Results indicated that SBH effectively scavenged ABTS, DPPH and SOD free radicals with IC50 values of 22, 36.15, and 79.85 v/v, respectively. SBH exhibited anti-proliferative activity against MCF-7 and HeLa cell lines, with IC50 values of 58.11 and 66.68 v/v, respectively. LCMS analysis, shows the presence of methyl syringate, 2-hydroxycinnamic acid, and fumaric acid in SBH which were not present in IBH. Docking experiments determined that these compounds, with the exception of fumaric acid, interacted stronger with the binding sites of ER β than ER α. The comprehensive findings from antioxidant, anti-proliferative, and docking studies underscore the potential anti-cancer properties of Tetragonula travancorica honey, particularly its heightened cytotoxicity against reproductive system cancer cell lines, such as of the breast and cervix.

Keywords

Anti-proliferative, estrogen receptor, stingless bee, cytotoxicity.

INTRODUCTION

Cancer is a devastating illness, claiming the lives of ten million people annually across the globe. It is currently the second most common cause of death globally, after cardiovascular disorders. According to recent estimates from the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO), there were approximately 10.0 million deaths from cancer globally in 2020, along with 19.3 million new cancer diagnoses. Most frequent cancers to be diagnosed were non-melanoma skin cancer with 1.20 million cases, stomach cancer with 1.09 million cases, colon and rectum cancer with 1.93 million cases, lung cancer with 2.21 million cases and female breast cancer with 2.26 million cases. [1].

Honey made from collected and modified plant exudates served as a prized natural dietary supplement since ancient times, renowned for its myriad medicinal and nutritional properties [2]. Natural honey's composition varies according to variables such as geographic regions, the sources of honeybee feeding, weather conditions, climate, and any treatments it may undergo [3] [4]. A variety of biological and chemical characteristics are present in honey, including its antioxidant [5], anti-inflammatory [6], anti-tumour [7] antimicrobial [8], bacteriostatic, wound and sunburn healing [9], anti-ulcer [10] and antiviral [11] activities.

A large number of bees known as stingless honey bees are members of the tribe Meliponini. They are members of the Apidae family, which includes other common bees. They are found throughout the majority of tropical and subtropical parts of the world, including Australia, Southeast Asia, Africa, and tropical America [12]. Surprisingly, although having better nutritional and therapeutic characteristics than other honey bee products, SBH is used less frequently as a dietary and medical supplement [13]. Stingless bee products are thought to be more promising sources of biologically active substances than honey bee products, because of the availability of plants in stingless bee habitats in the tropics and subtropics. In several populations in South America and Africa, stingless bee honey is utilized as medicine. SBH has been demonstrated to have therapeutic potential in hyperglycemia, the healing process, vision problems, blood pressure, reproductive problems, malignancies, chronic infections and disrupted lipid levels, which could be due to the different compounds present in the honey [14]. The bee of interest *Tetragonula travancorica* is a new species distinguished from other Indian species of the subgenus. This species has morphological changes from *Tetragonula iridipennis*, which is predominantly found in India but was originally discovered on the island of Sri Lanka [15]. It can

be distinguished from *Tetragonula iridipennis* by a change in its hind wings, longer antenna and other features [16]. But these species are poorly characterized and have not been directly compared with molecular studies. Hence, there is uncertainty about whether they are separate species [17].

OBJECTIVE

The purpose of this study was to evaluate the potential anticancer properties of stingless bee honey, recognizing its significance. A comparative evaluation was conducted with Indian bee (*Apis indica*) honey. The study involved assessing its cytotoxic potential on murine tumour cells such as DLA and EAC and examining the antiproliferative effects on various cancer cell lines of human origin, including breast and cervix. Using assays for ABTS, DPPH and SOD radical scavenging, its antioxidant properties were evaluated. Compounds of the honeys were qualitatively analyzed through the LCMS method and the compounds identified were docked using *in silico* methods.

MATERIAL AND METHODS

Collection and preparation of honey samples

Stingless bee honey (SBH) and Indian bee honey (IBH) were sourced from College of Agriculture, Thiruvananthapuram, affiliated with Kerala Agricultural University. The samples were kept under sunlight and filtered using a muslin cloth. The honey was prepared in an aqueous solution as volume/volume.

Cell lines

Cervical cancer cells, HeLa; breast cancer cells MCF-7; Intestinal epithelial cells, IEC-6 and kidney epithelial cells, Vero were sourced from the National Centre for Cell Science, Pune. The cell lines were maintained at 37°C, 5% CO2, 100% humidity, 95% air and were cultivated in DMEM medium supplemented with 10% v/v FBS, 100 μg/mL antibiotic and 100 U/mL penicillin. The Ehrlich Ascites Carcinoma (EAC) and Daltons Lymphoma Ascites (DLA) cell lines were acquired from the Amala Cancer Research Centre, Kerala.

Total phenolic content

The Folin-Ciocalteu colorimetric technique was utilized to evaluate the phenolic content [18]. In this analysis, a 50 μL aliquot of the extract was combined with 1 mL Folin-Ciocalteu phenol reagent and 0.8 mL 7.5% sodium carbonate. Following a 60-minute incubation at room temperature, the absorbance of mixture at 765 nm was measured using a Systronics UV-VIS Spectrophotometer Type 119. Gallic acid equivalents (GAE), measured in milligrams per gram of extract, were used to express the results.

Total flavonoid content

Aluminium chloride colorimetric technique was employed to evaluate the flavonoid concentration. [19]. 0.1 mL of plant extracts were combined with 1 mL 2% aluminium chloride. A separate test tube containing a blank was prepared. These tubes were incubated at room temperature for 30 minutes, and the absorbance at 415 nm was recorded. Quercetin equivalents (QTE) measured in milligrams per gram of extract were used to express the total flavonoid concentration.

In vitro antioxidant assays

DPPH radical scavenging assay

To evaluate the extract's ability to scavenge free radicals, the DPPH technique was employed [20]. Various extract concentrations were introduced into 187 μL of newly made DPPH solution. Methanol was added to the volume to get it up to 1000 μL. The absorbance at 517 nm of the reaction mixture was measured after 20 minutes of dark incubation. The following formula was used to get the radical scavenging percentage: % scavenging of DPPH = Abs. of blank - Abs. of sample / Abs. of blank x 100.

ABTS radical scavenging assay

The ABTS assay was used to evaluate the samples' ability to scavenge free radicals [21]. A 1:1 reaction between ABTS in water (7 mM) and potassium persulfate (2.45 mM) yielded the ABTS radical. An equal quantity of methanol was mixed, and it was let to stand at room temperature for 12 to 16 hours in dark before being utilized. After that, methanol was added to the ABTS•+ solution to dilute it until the absorbance at 734 nm was between 0.700 and 0.800. Thirty minutes after the first mixing, the absorbance was measured after adding one to five microliters of samples and making up the reaction volume to four milliliters with diluted ABTS•+ solution. For every assay, a suitable solvent blank was run. A minimum of three measurements were made for each. The calculation of percentage inhibition of absorbance at 734 nm was determined using the following formula: % scavenging of ABTS = Abs. of blank - Abs. of sample / Abs. of blank x 100.

Superoxide scavenging assay

The procedure of nitro blue tetrazolium (NBT) reduction was utilized to ascertain the extracts' ability to scavenge superoxide radical [22]. It is dependent upon riboflavin's production of superoxide when exposed to light and NBT's subsequent decrease. The reaction mixture (3 mL) contained EDTA (0.1 M), NaCN (0.3 mM), riboflavin (0.12 mM), NBT (1.5 mM) and phosphate buffer (0.067 M), supplemented with different quantities of the plant extracts. An incandescent light was used to illuminate the tubes uniformly for 15 minutes. The optical density was determined at 560 nm both prior to and following the light exposure. The absorbance values of the control and experimental tubes were compared in order to determine the percentage reduction of superoxide production.

Trypan blue dye exclusion method

Using the trypan blue dye exclusion method, the short-term cytotoxicity of SBH and IBH was assessed using murine tumor cells, DLA and EAC cells [23]. Trypsin was used to treat the cells, and after being washed with PBS, they were centrifuged for five minutes at 1000 rpm. Upon resuspending

the cell pellets in PBS, $1x10^6$ cells/mL was used as the corrected cell count. After pipetting out the cells, they were transferred into separate PBS tubes containing various doses of samples. After that, the tubes were incubated at 37 ºC for three hours. Trypan blue dye was added after the incubation period, and there was a three-minute waiting interval. Lastly, a hemocytometer was used to examine the cells under a microscope. By calculating the ratio of all dead cells to all living cells and entering these numbers into the calculation, the percentage of cytotoxicity was determined:

$$\% \text{ of cytotoxicity} = \frac{\text{No. of dead cells}}{\text{Total no of cells}} \times 100$$

The Hill equation was employed to fit the dose-response curve of SBH and IBH [24].

MTT assay

In 24 well plates with medium, the MCF-7, HeLa, Vero, and IEC-6 cell lines were added (0.5 x 105 cells) and incubated at 37 ºC for 24 hours. Following that, cells were cultured for 48 hours at 37 ºC with various doses of SBH and IBH. Additionally, a blank containing a full culture media devoid of cells was included in the test. Following incubation, well plates were transferred to the new medium and rinsed with phosphate buffer saline (PBS). Each well received 50 µL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), which was then incubated at 37 ºC for four hours. One mL of DMSO was used to dissolve the dark blue formazan crystals through repeated mixing and re-suspension. The absorbance of the resulting colored product was recorded at 570 nm. Cytotoxicity was assessed by comparing the proportion of dead cells in the treated population of cells to untreated cells as shown by their relative absorbance levels determined using the MTT test (Mosmann, 1983). The Hill equation was used to fit SBH and IBH's dose-response curves [24].

$$\frac{E}{Emax} = \frac{1}{1+\left(\frac{EC50}{|A|}\right)^n}$$

Where the maximum percentage of inhibition is E_{max}, the half-maximal effective concentration is EC_{50}, the Hill coefficient is n and the drug concentration is A. The hill equation was calculated with data analysis and graphing software, OriginPro 9.

UV-VIS spectrum analysis

The UV-VIS spectrum analysis of honey samples was done by diluting with water and scanned at a wavelength ranging from 200 to 500 nm using UV-VIS Spectrophotometer Type T80+ (PG Instruments Ltd). The characteristic peaks were detected (White Jr, 1979). Using the following formula, the hydroxymethylfurfural (HMF) amount in honey was determined:

$$\text{HMF}\left(\frac{\text{mg}}{100\text{g}}\right)\text{honey} = \frac{(A284-A336)\times 74.87}{W}$$

Where W is the weight of the sample (g), A284, A336= absorbance reading at 284 and 336 nm.

LCMS analysis

Honey chromatographic separation was conducted at CARe Keralam, KINFRA Park, Thrissur, using the Agilent Technologies 1260 Infinity MS-6120 Quadrupole instrument with an Agilent Eclipse Plus C-18 column (4.6 x 250 mm). The mobile phase was composed of a 60:40 ratio of acetonitrile to 0.1% acetic acid in water and 10 μL injection volume was utilized, employing a 0.6 mL/min flow rate, leading to a total run time of 42 minutes.

Molecular docking

To investigate the possible interactions between specific compounds identified in stingless bee honey (methyl syringate, 2-hydroxycinnamic acid, fumaric acid) with the targeted estrogen receptor (ER) α and estrogen receptor (ER) β, molecular docking was done using Schrodinger Maestro software.

RESULTS

Collection and preparation of honey

The stingless bee honey appeared darker than Indian bee honey and the viscosity of SBH was observed less compared to IBH. The microscopic evaluation of SBH showed pollen grains of various shapes and IBH did not show any presence of pollen grains.

Total phenol and flavonoid content estimation

The total phenol content of stingless bee and Indian bee honey were 1.591 and 1.22 mg GAE/g extract and 0.266 and 0.242 mg QE/g extract respectively.

In vitro antioxidant assays

The DPPH and ABTS free radical were scavenged by SBH and IBH with an IC_{50} value of 36.15, 72.75 v/v and 22, 57.89 v/v respectively. The superoxide radical was scavenged by SBH with an IC_{50} of 79.85 v/v. For IBH there was 44 % inhibition observed at a concentration of 100 v/v (Figure 1).

Trypan blue dye method

With IC50 values of 37.41 and 48.02 v/v, respectively, the stingless bee honey revealed considerable cytotoxic activity towards EAC and DLA cell lines in a concentration-dependent way. The Indian honey has shown barely 2.56 and 3.45 % cytotoxicity for EAC and DLA cell lines, respectively, at the highest dosage of 100 v/v.

MTT assay

It was discovered that the stingless bee honey was cytotoxic to HeLa and MCF-7 cell lines with IC50 values of 66.68 and 58.11 v/v, respectively (Figure 1 and 2). Different concentrations of IBH were tested for cytotoxicity and found to have 24.79 and 37.35 % inhibition at a concentration of 100 v/v for HeLa and MCF-7 respectively. The *in vitro* safety evaluation done in Vero and IEC-6 did not show any cytotoxicity up to the concentration of 100 v/v (Figure 1and 2).

International Journal of Agro Nutrifood Practices
Vol-3, Issue-4, December 2023
e-ISSN: 2583-066X

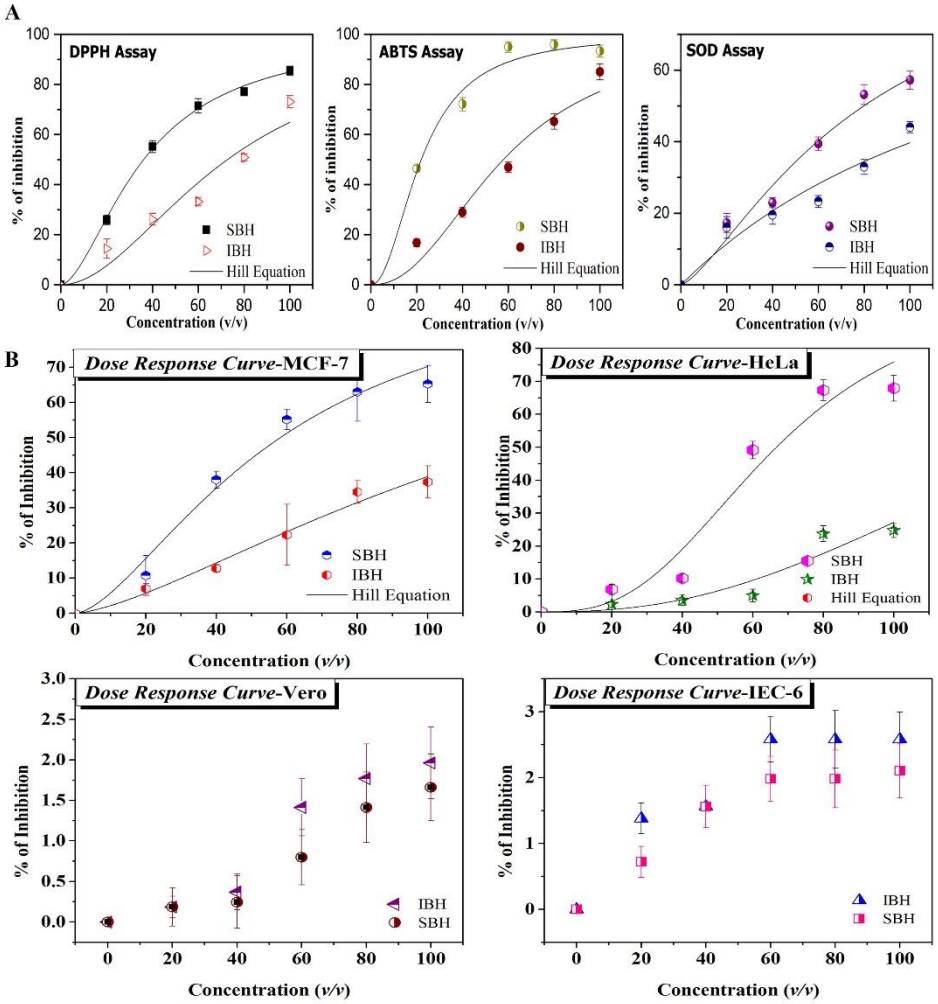


Figure 1 (A) Inhibition percentage (%) of free radicals by SBH and IBH (B) Inhibition percentage (%) of different cell lines after honey treatment

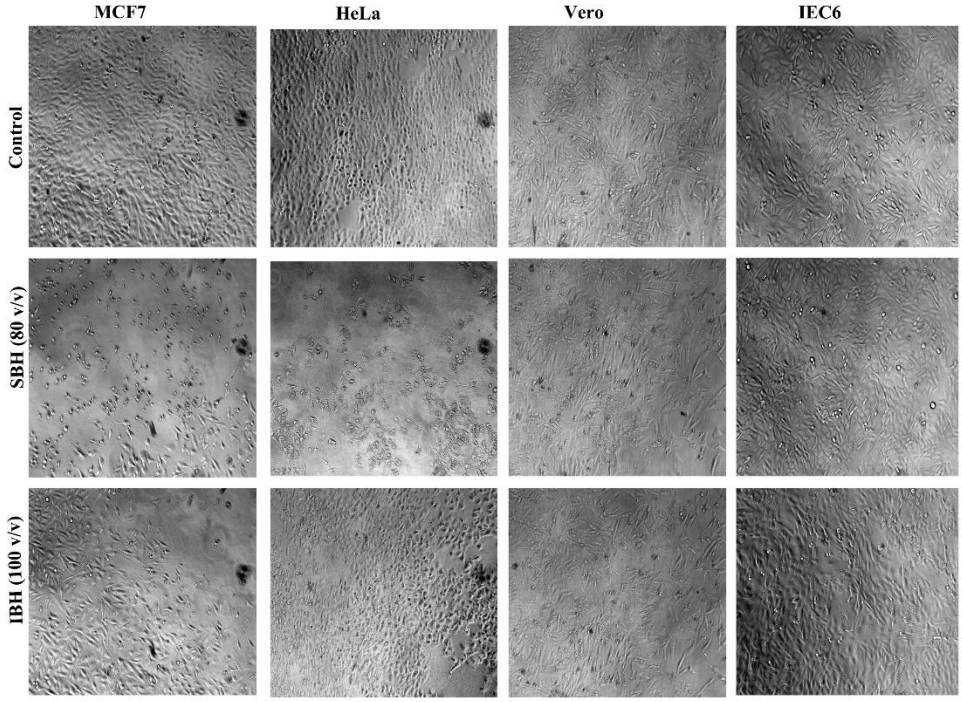


Figure 2. Morphology of different cell lines after honey treatment

UV-VIS spectrum analysis

The UV-VIS spectrum profile of SBH and IBH showed peaks between 200-500 nm. In the spectra of SBH, excitations were observed at 220, 284, and 384, whereas IBH exhibited peaks at 280 and 344, accompanied by a few minor peaks (Figure 3). The peaks obtained at 284 nm are of hydroxymethylfurfural (HMF). Its weight ratio was calculated and found to be 14.30 and 8.19 mg/kg. The HMF amount was seen more in SBH compared to IBH.

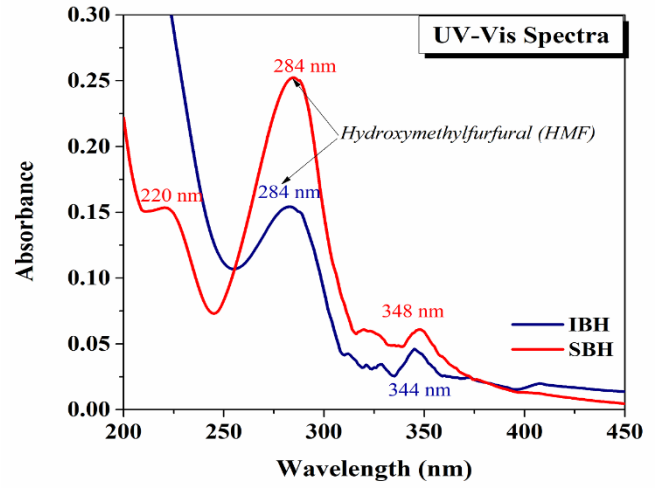
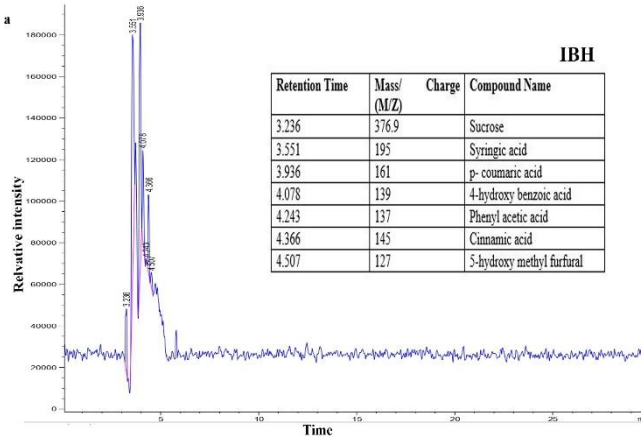


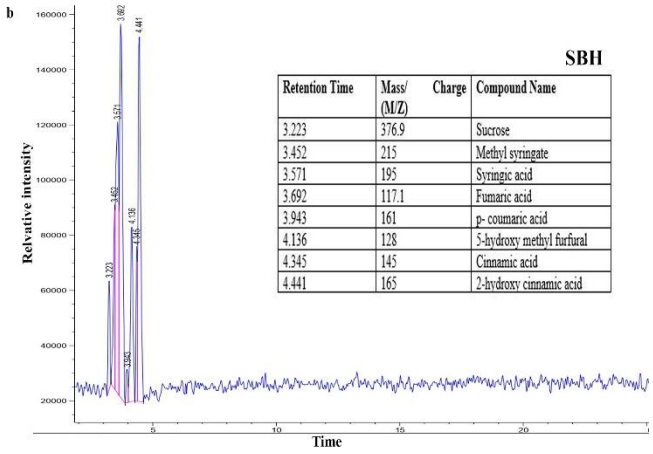
Figure 3. UV-VIS spectra of SBH and IBH

LCMS analysis

The SBH contains sucrose, methyl syringate, syringic acid, fumaric acid, p- coumaric acid, 5-hydroxy methyl furfural, cinnamic acid and 2-hydroxy cinnamic acid whereas IBH contains constituents such as sucrose, syringic acid, phenylacetic acid, 4-hydroxy benzoic acid, p- coumaric acid and 5-hydroxy methyl furfural (Figure 4). The stingless bee honey was found to have specific compounds such as methyl syringate, fumaric acid and 2-hydroxy cinnamic acid with the retention time of 3.452, 3.692 and 4.441 respectively.



Retention Time	Mass/ Charge (M/Z)	Compound Name
3.236	376.9	Sucrose
3.551	195	Syringic acid
3.936	161	p- coumaric acid
4.078	139	4-hydroxy benzoic acid
4.243	137	Phenyl acetic acid
4.366	145	Cinnamic acid
4.507	127	5-hydroxy methyl furfural



Retention Time	Mass/ Charge (M/Z)	Compound Name
3.223	376.9	Sucrose
3.452	215	Methyl syringate
3.571	195	Syringic acid
3.692	117.1	Fumaric acid
3.943	161	p- coumaric acid
4.136	128	5-hydroxy methyl furfural
4.345	145	Cinnamic acid
4.441	165	2-hydroxy cinnamic acid

Figure 4. (a, b). LCMS spectrum of SBH, IBH and its constituents

Molecular docking

The docking of the inbuilt ligand, estradiol into the 3D structure estrogen β (3OLS) was done using a glide dock. The active site of 3OLS comprises the following amino acid residues: LEU 390, PHE 377, ILE 376, ILE 373, LEU 339, MET 340, MET 336, ALA 302, ARG 346, LEU 343, GLU 305, PHE 356, LEU 301, LEU 298, THR 299, MET 295, MET 479, HID 475, LEU 476 and GLY 472. The docking data gave additional insights into the selective interactions of estradiol with 3OLS in a 2D picture, as shown in Figure5. Estradiol, the inbuilt ligand, was bound deep into the active site area, making weak hydrogen bonding interactions with HID 475, ARG 346, GLU 305 and π-π stacking interactions with PHE 356. The inbuilt ligand shows a docking score of -11.635 and binding energy of -94.07 kcal/mol (Tab.1). After being docked into the active site region, the 2-hydroxy cinnamic acid interacted with the residues through hydrogen bonding with LEU 298 and π-π stacking with PHE 356 (Figure5). The docking score and binding energy were found to be -6.146 and -35.607 kcal/mol which was more compared to 5- hydroxy methyl furfural, methyl syringate and fumaric acid (Tab. 1).

The estradiol was docked into the 3D structure estrogen α (1A52). PHE 434, LEU 349, MET 528, THR 347, ALA 350, LEU 346, MET 343, LEU 525, HIE 524, GLY 521, MET 421, ILE 424, LEU 384, TRP 383, LEU 428, LEU 387, MET 388, ARG 394, LEU 391 and GLU 353 are residues of amino acids that can be found in 1A52's active site. After the estradiol was docked into the active site region, it interacted with the residues through π-π stacking with PHE 434, hydrogen bonding with ARG 394, GLU 353, and HIE 524 (Figure 5 and 6). The inbuilt ligand shows a docking score of -10.917 and binding energy of -89.185 kcal/mol (Tab. 1). The other ligand, 2-hydroxy cinnamic acid docked into active sites, made interactions with the residues by π-π stacking with PHE 356 and hydrogen bonding with LEU 298 (Figure 5 and 6). The docking score and binding energy were found to be 4.955 and -41.58 kcal/mol which was better compared to 5-hydroxy methyl furfural, methyl syringate and fumaric acid.

International Journal of Agro Nutrifood Practices
Vol-3, Issue-4, December 2023
e-ISSN: 2583-066X

Table 1. Binding energy and docking score of estrogen receptors and ligands

Chemical Compounds	Estrogen alpha		Estrogen beta	
	Docking Score	Binding Energy	Docking Score	Binding Energy
Inbuilt ligand (Estradiol)	-10.917	-89.185	-11.635	-94.07
2-hydroxy cinnamic acid	-4.955	-41.58	-6.146	-35.607
5-Hydroxymethylfurfural	-4.587	-33.614	-5.905	-36.151
Methyl syringate	-4.09	-31.72	-5.950	-44.314
fumaric acid	-1.813	-13.106	-3.187	-20.747

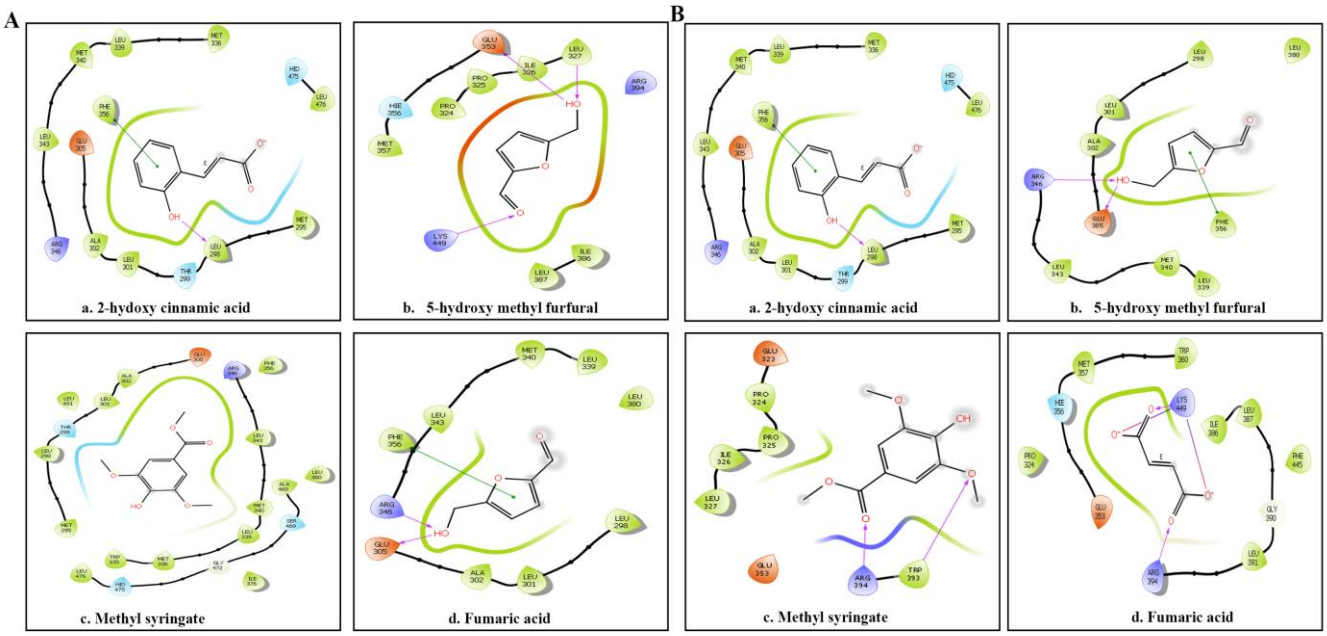


Figure 5. (A) Two-dimensional image of the interaction between estrogen receptor β and ligands (B) estrogen receptor α and ligands

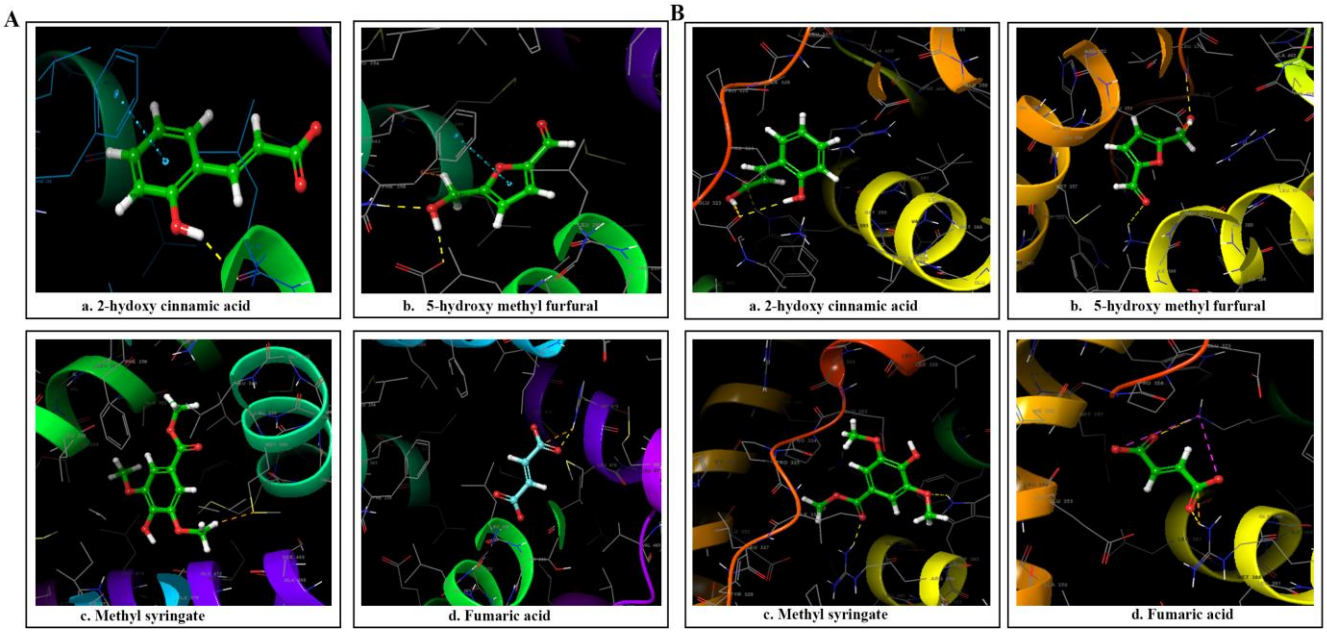


Figure 6. (A) Three-dimensional image of the interaction between estrogen receptor β and ligands (B) estrogen receptor α and ligands

DISCUSSION

The stingless bee honey showed significant antiproliferative activity on breast and cervical cancer cell lines and cytotoxicity towards murine tumour cell lines such as DLA and EAC than Indian bee honey suggesting its anticancer potential. Stingless bee honey exhibits an enhanced ability to scavenge free radicals such DPPH, ABTS, and superoxide than Indian bee honey, demonstrating its antioxidant properties. Fumaric acid, methyl syringate, and 2-hydroxy cinnamic acid were detected in SBH by LCMS analysis; these compounds were absent from IBH. Also in molecular docking, compounds identified in SBH bind well with estrogen receptor β than estrogen receptor α.

Stingless bee honey demonstrated superior scavenging of DPPH, ABTS, and superoxide free radicals compared to Indian bee honey. Studies have also documented the antioxidant capacity of several varieties of honey produced by stingless bees. For instance, Australian pot-honey from *Tetragonula carbonaria* has exhibited DPPH and ABTS free radical scavenging activity [25]. An additional study involving *Tetragona clavipes* and *Trigona fuscipennis* honey revealed DPPH and FRAP radical scavenging activity [26]. The ability to scavenge radicals is linked to the capacity of secondary metabolites to counteract free radicals and other reactive species within the body [27]. Consequently, it is presumed that the polyphenols present in stingless bee honey play a pivotal role in its antioxidant properties. These compounds have a potent scavenging effect on free radicals. There is a positive relationship between the presence of polyphenols and the effectiveness of antioxidants, as phenolic and flavonoid compounds are closely related to antioxidant activity [28].

The stingless bee honey revealed significant cytotoxic activity toward breast cancer and cervical cancer cell lines. Upon SBH treatment, the morphology of cell lines was changed from an epithelial-like appearance to a round shape. The cells were shrunk and some granulation was also found in the cells. Many cells were detached from the surface and seen to be floating in the media. In normal cell lines, Vero and IEC-6 did not show any cytotoxicity up to the concentration of 100 v/v. Additionally, stingless bee honey's cytotoxic properties have been documented by other authors. The cytotoxic activity of *T. laeviceps* honey, an Indonesian stingless bee, was examined *in vitro* on the HepG2 liver cancer cell line [29]. The anti-cancer potential of sixteen kinds of pot honey produced by thirteen different stingless bee species was assessed using human ovarian cancer cells. The findings indicated significant variations in the ability of honey samples to induce cancer cell death [30]. Moreover, using the trypan blue exclusion method, it was observed that SBH exhibited substantial cytotoxicity towards murine tumour cells, including the DLA and EAC cell lines, in a dose-dependent manner. These preliminary studies, taken together, reveal promising results that suggest SBH could be used as cancer therapeutics. This unique activity could be owing to the polyphenols present, and they could do so through specific mechanisms including gene regulation or altering metabolic pathways in cancer cells [31]. While there are reports on the anti-proliferative properties of stingless bee propolis and cerumen [32, 33], there hasn't been much research on stingless bee honey's anticancer properties, which calls for more research.

The LCMS analysis of SBH identified some compounds which were not present in IBH and they include 2-hydroxy cinnamic acid, methyl syringate and fumaric acid. The first two are plant phenolic compounds and the latter is an organic acid naturally present in honey. Polyphenols have been found in honey produced by the *Tetragonula* genus, according to reports by various authors. The LCMS analysis of *Tetragonula carbonaria* honey from Australia identified quercetin, kaempferol and isorhamnetin [34]. The Indonesian stingless bee, *Tetragonula laeviceps* honey contains 17 amino acids detected by LCMS/MS [35]. As reported by another author, LC-MS analysis has revealed that *Tetragonula biroi* honey contains the flavonoid isorhamnetin (3-methylquercetin) [36]. The primary polyphenolic groups found in honey are these flavonoids and phenolic acids, which are derived from benzoic and cinnamic acids. Both SBH and IBH have the presence of flavonoids and phenols with SBH having more amounts. Polyphenols present in honey come through flower nectar, propolis and pollen. These compounds have various nutritional properties and have a possible role in treating various diseases [9].

Molecular docking was employed to explore potential interactions between the specific compounds present in SBH and targeted receptors, namely estrogen receptor (ER) α and estrogen receptor (ER) β, within MCF7 and HeLa cell lines. ERα plays a significant role in differentiation by controlling target genes, leading to proliferation. In contrast, ERβ serves as a potent tumour suppressor inhibiting the growth of cells and mitigating ERα's impact in reproductive tissue across various cancer types [37]. The results of docking of 2-hydroxy cinnamic acid with ERβ show interactions by hydrogen bonding with LEU 298 and π-π stacking with PHE 356. The ligand shows the highest docking score of -6.146 and binding energy of -35.607 kcal/mol compared with other ligands such as methyl syringate and fumaric acid. Also, all the ligands bind well with estrogen receptor β than estrogen receptor α. This could be the cause of stingless bee honey's anticancer properties, as ligand binding to ERβ leads to inhibition of cell proliferation. In a study using the Schrödinger Maestro v10.1 program, molecular docking performed with six active honeybee products from manuka honey with SARS-CoV-2 Mpro showed good binding affinity and the outcomes suggested that it might prevent the COVID-19 virus from replicating [38]. According to a recent study, phenylalanine, a constituent of Malaysian stingless bee honey, has the ability to cause the overexpression of synaptic genes, such as brain-derived neurotrophic factor and inositol 1,4,5-triphosphate receptor type 1 [39]. According to the author, in-depth investigations into the docking studies,

quantum-chemical analysis and antioxidant characteristics of the main phenolic components found in honey of stingless bee are still necessary. Hence, several studies are reporting the potent biological compounds present in stingless bee honey and computational methods should be done to analyse its binding to active receptors, as *in-silico* methods have an important role in drug designing.

CONCLUSION

Stingless bee honey has higher antioxidant activity and is shown to have significant antiproliferative activity against cancers of reproductive system including cervical and breast cancer, than Indian bee honey. The characterization revealed some additional compounds in the honey of stingless bees that were absent from Indian bee honey. Also, the *in-silico* studies revealed the effective binding of compounds found in SBH to ERβ than ERα which might the reason for its anticancer activity. Given its superior nutritional and therapeutic attributes compared to other honey bee products, further research into the biological activities of stingless bee honey is necessary.

DECLARATIONS

- Acknowledgements-The first author extends thanks to the Department of Entomology and Department of Agricultural Biotechnology at the College of Agriculture, Thiruvananthapuram, as well as the Department of Biochemistry at the Amala Cancer Research Centre for their assistance and the facilities they made available for the study.
- Ethical statements (and/or Informed Consent in case of human study)- not applicable
- Conflict of competing interest - The authors all agree that the data should be published, and they all declare that they have no conflicting interests.

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