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Screening and assessment of molecular mechanistic actions of 5-hydroxy-1-methylpiperidin-2-one against free radicals, lung cancer cell line (A549), and binding properties on bovine serum albumin

Sangilimuthu Alagar Yadav[1*], Lukmanul Hakkim Faruck[2,3*], Rajagopal Subramanium[4], Lakshmi K. Surendren[5] and Hamid Bakshi[6]

Abstract

Background: Natural products play a key role in treating different ailment including diabetes, asthma, skin diseases, and cancer. It is well known that synthetic drugs elicit significant toxicity when used in the clinic. A higher drug affinity towards carrier protein Bovine Serum Albumin (BSA) would enhance a higher drug bioavailability which in turn leads to a higher therapeutic efficacy. The focus of the present study was to investigate antioxidant and anti-cancer potential of 5-hyrdoxy1-methylpiperidin-2-one (5-HMP) isolated from leaves of *Tragia involucrata*.

Methods and material: In vitro free radical scavenging assays and MTT assay were employed to assess the antioxidant activity of 5-HMP and cytotoxicity of 5-HMP on lung cancer cell line, A549, respectively. In addition, attempts were made to investigate 5-HMP binding capacity on BSA by spectral studies and molecular docking.

Results: The antioxidant data revealed that 5-HMP inhibited the radicals with an IC_{50} value of 49.55 ± 0.75 μg/ml which was comparable with the IC_{50} values afforded by L-ascorbic acid. 5-HMP exhibited a dose-dependent cytotoxicity on A549 cells with an IC_{50} value of 30.00 ± 0.55 μg/ml. further 5-HMP induced a cell cycle arrest in A549 at S and G2/M phase. The fluorescence quenching was observed when an increasing concentration of 5-HMP, reacts with a fixed concentration of BSA (1.0 μM). The fluorescence quenching of BSA by 5-HMP indicated a binding constant of $K_{5\text{-HMP}} = 2.8 \pm 1.4 \times 10^4 M^{-1}$ with corresponding binding free energy (ΔG)−6.06 K.cal/mole.

Conclusions: This paper concluded that 5-HMP possesses antioxidant properties, cytotoxic effects and also it possesses good drug binding properties on bovine serum albumin.

Keywords: 5-hydroxy-1-methypiperidin-2-one, *Tragia involucrata*, Antioxidant, Cytotoxicity, Drug-binding properties, Bovine serum albumin, Docking analysis

* Correspondence: smuthu.al@gmail.com; clonehakkim@gmail.com
[1]Department of Biotechnology, Karpagam Academy of Higher Education, Eachanari - Post, Coimbatore 641021, Tamil Nadu, India
[2]Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Salalah, Oman
Full list of author information is available at the end of the article

Key messages

This paper provides a novel phyto-molecule as 5-hyrdoxy-1-methylpiperidin-2-one (5-HMP) from *Tragia Involucrata* leaves as good antioxidant, cytotoxic agent on lung cancer cell line and good drug binding properties on BSA.

Background

Cancer is the largest and second leading cause of death globally that accounted for 8.8 million deaths. Worldwide, the lung cancers are top most type of cancer that affect people. As per the American Cancer Society, 121,680 have been diagnosed for lung cancer in 2018 and 83,550 of them would die due to inefficient therapies [1]. Based on the recent WHO report, breast cancer, colorectal cancer, and lung cancer, and the fourth leading affecting cancer which is the cervical cancer among women, are the leading types of cancers [2]. Chemotherapy, radiotherapy, and surgery are the most widely used strategies in lung cancer treatment. However, standard chemotherapies elicit severe toxicity for patients and may result in limited survival benefit. Further, multidrug resistance is the major limitation of lung cancer treatment. Free radicals are unstable and can bind to proteins, lipids, and DNA in the cells and associated with various diseases such as cancer, diabetics, and aging [3]. Lungs are significantly exposed to free radicals because of the role they fulfil. High oxygen pressure, comparable to atmospheric values, promotes oxidation, particularly in the presence of reactive oxygen species (ROS) from tobacco smoke and air pollution [3]. Oxidative stress plays an important role in lung cancer pathogenesis; therefore, protection from ROS seems to be one of the crucial strategies of lung cancer prevention [4]. Current attention on the use of herbal medicines from plant sources has been the hot topic in drug development in cancer treatment for years due to their effectiveness in eradicating different cancers. Indeed, traditional healing strategies around the world have utilized herbal remedies as an important source for the discovery of new antibiotics and drugs such as vincristine and vinblastine against small cell lung cancer [5, 6]. As per WHO, nearly 80% of the population relies on traditional medicine for their primary health care needs.

Tragia involucrata Linn. (Family Euphobiaceae) is a shrub widely distributed in the Indian subcontinent. It grows aggressively as a dry land weed. The tribes in Western Ghats of India use different parts of this plant for the treatment of inflammation, wounds, and skin infections [7]. The efficacy of this plant is well known by Indian traditional medicine experts in the treatment of inflammation, wounds eczema, and headache [8]. Furthermore *T. involucrata* has been reported to induce nephro protective [9], anti-fertility [10], antioxidant [11], anti-diabetic [12], hepatoprotection [13], and cytotoxicity effects [10]. Recently, we reported anti-histamine property of 5-HMP which is isolated from *Tragia involucrata* leaves [14]. 5-HMP is a novel molecule from natural source and this is the first report of 5-HMP against free radicals and lung cancer cell line (A549). The blood components influence the bioavailability of drugs which will in turn affect their stability and induced toxicity on tumors [15–18]. Albumin proteins contribute to the osmotic pressure as well as playing a vital role in the drug distribution and efficiency [19, 20]. Indeed, in the circulatory system, the albumin proteins are major soluble and they play a vital role in the biological system [21]. Small molecules binding on serum albumin makes protein-ligand complexes, which are preliminary step of drug's (adsorption, distribution, metabolism, and excretion) ADME features [22]. Bovine serum albumin (BSA) as a binding protein has been extensively characterized. The structure of BSA is 76% similar to human serum albumin (HSA) [23]. A BSA solution is stable and homogeneous. BSA has been one of the most widely deliberate of this set of proteins, mainly because of its structural homology with HSA. Further serum albumin increases the solubility of hydrophobic drug in plasma and induces a conformational change in the structure of the drug. This would favor a more specific binding to a receptor protein. Binding studies have shown an interaction between small molecules on the active site of the macromolecule protein. These interactions can be observed using various spectral analysis such as UV-Visible spectral analysis [24], Fourier transform infrared spectral analysis, HPLC [25], fluorescence spectral studies, and circular dichroism spectroscopy (CD). BSA has 69,000 KD molecular weight with 2 tryptophan and 20 tyrosine amino acid residues as fluorescence emitting residues [26]. Indeed, numerous previous studies have investigated the interaction of small molecules with BSA, HSA, and DNA [27–30] for their binding efficacy. Keeping these facts in mind in this study, we explored the binding property of 5-HMP on BSA by various spectroscopic and docking analysis in addition to antioxidant and anti-lung cancer activity of 5-HMP.

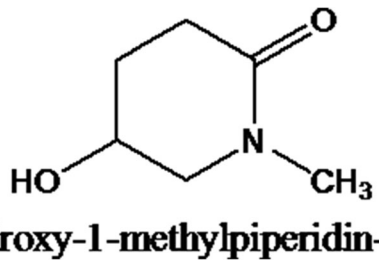


Fig. 1 Structure of 5-hydroxy-1-methylpiperidin-2-one. The molecular weight and molecular formula are 129.16 and $C_6H_{11}NO_2$ respectively

Table 1 IC_{50} values for DPPH and ABTS radical scavenging activity of 5-HMP and L-ascorbic acid

S. no.	Name of the compound	IC_{50} value (μg/ml) in DPPH assay	IC_{50} value (μg/ml) in ABTS assay
1	L-ascorbic acid	13.20 ± 1.25	13.20 ± 1.25
4	5-Hydroxy-1-methyl piperidine-2-one	49.55 ± 0.75	62.75 ± 1.25

Methods

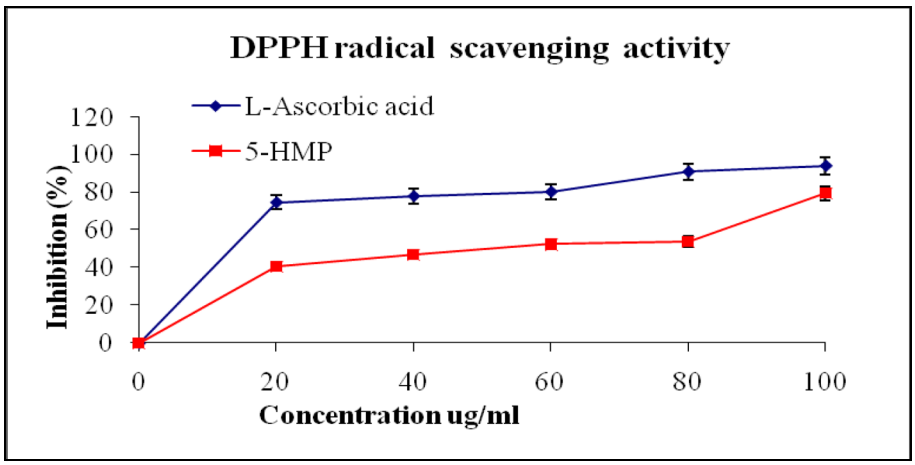
Isolation of 5-HMP from *T. involucrata*

The defatted alcoholic extract of *T. involucrata* L. leave (20 g) was fractionated by column chromatography with increase polar order of the solvents. Fraction 18–37 contains a single spot by TLC with 0.37 cm R_f value and characterized the molecular structure as 5-HMP (Fig. 1) using various spectral studies such as UV-V, FT-IR, and NMR as described earlier [14], that have been taken for antioxidant, anti-cancer activity, and drug-binding characteristic feature on BSA.

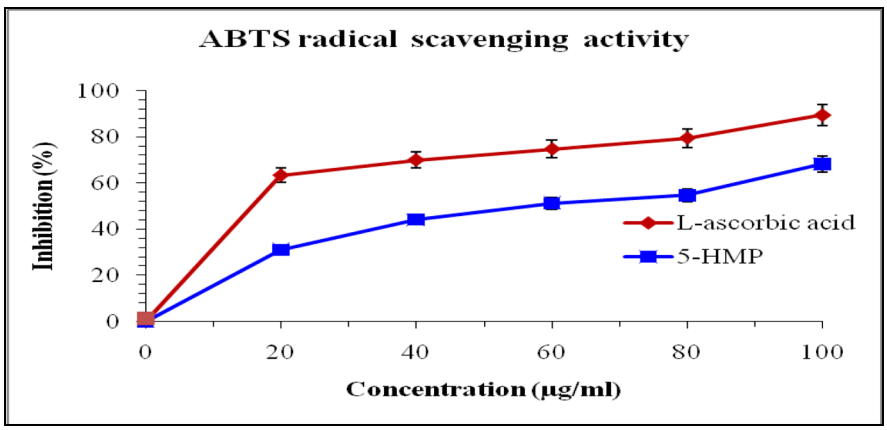
Biological properties of 5-HMP

DPPH free radical scavenging activity of 5-HMP

Experiments were carried out to investigate the ability of 5-HMP to scavenge DPPH radical. The method was described elsewhere [31]. Briefly, aliquot of the extract 20–100 μg/ml was treated with 3.0 ml DPPH. The colour changes were observed using UV-Visible spectrophotometer at 517 nm after 30 min incubation at room temperature indicated that the tested drug possesses an inhibiting activity against the free radicals. In the same



A: DPPH radical scavenging activity of 5-HMP



B: ABTS radical scavenging activity of 5-HMP

Fig. 2 a DPPH radical scavenging activity of 5-HMP. **b** ABTS radical scavenging activity of 5-HMP

way, ABTS radical scavenging ability of 5-HMP was performed [32] and calculated the percentage inhibition using the formula

$$\text{Percentage of inhibition } (\%) = [(A_{control} - A_{Sample})/A_{control} \times 100 \quad (1)$$

In vitro anti-cancer activity of 5-HMP

Human lung cancer cell line (A549) was purchased and maintained as per the procedure following Mosmann's (1983) [33]. Lung cell line was treated with various concentrations of 5-HMP (6, 12, 25, 55, and 85 μg/ml) as per our earlier report [34]. The inhibition of 5-HMP was calculated the following formula: % Cell Inhibition = 100−Abs (sample)/Abs(control) × 100

The DNA content was measured and the cell cycle arrest in the lung cancer cell line was observed after the treatment of 5-HMP by using the flow cytometry (FACS, BD Bioscience).

Binding properties of 5-HMP on bovine serum albumin

Preparation of protein and ligand

Fat-free bovine serum albumin was purchased from Aldrich chemical Pvt Ltd., and was dissolved in phosphate buffer (1.0 mM) with pH 7.4. BSA, and ligand was prepared as per our earlier report [4, 15].

Fluorescence spectroscopy, displacement, and synchronous studies of protein-ligand complex

Fluorescence quenching mechanism and free energy of 5-HMP on BSA was determined followed by our earlier report (Yadav et al. 2018). The quenching and binding constant was achieved by using the stern-volmer plot with following formula 2.

$$\text{Log}[F_0 - F] = \log K_s + n\text{Xlog}[Q] \quad (2)$$

Displacement test of BSA-5-HMP complex with site exact markers (phenylbutazone-site I, Ibuprofen-site II, Lidocaine−site IB) was followed. Binding location was confirmed by molecular docking studies (BSA (PDB ID: 1A06) and 5-HMP with Autodock tool. The synchronous and micro-environment changes of BSA–5-HMP were recorded (Δλ 15 nm, 60 nm, and 90 nm) [35].

UV-Visible spectrophotometer analysis

UV-Vis spectral observation of protein-ligand complex conformation is a simple and cost-effective method to the structural changes and conformation of complex formation; this technique measures the interaction of BSA and ligand complex with light energy range between 150 and 400 kJ mol to promote electrons from the ground state to excited state. The absorption spectra of different concentrations of 5-HMP (0.01, 0.025, 0.050, 0.075, and 0.1 mM) at a fixed concentration of BSA (0.01 mM) were recorded in the range of 250–350 nm by Perkin Elmer UV/Visible spectrophotometer Lamda 35 [36].

Results

Biological properties of 5-HMP

DPPH is commonly used for assessing the antioxidant effect of molecules or extracts. Table 1 and Fig. 2a illustrate the DPPH radicals scavenging activity of 5-HMP

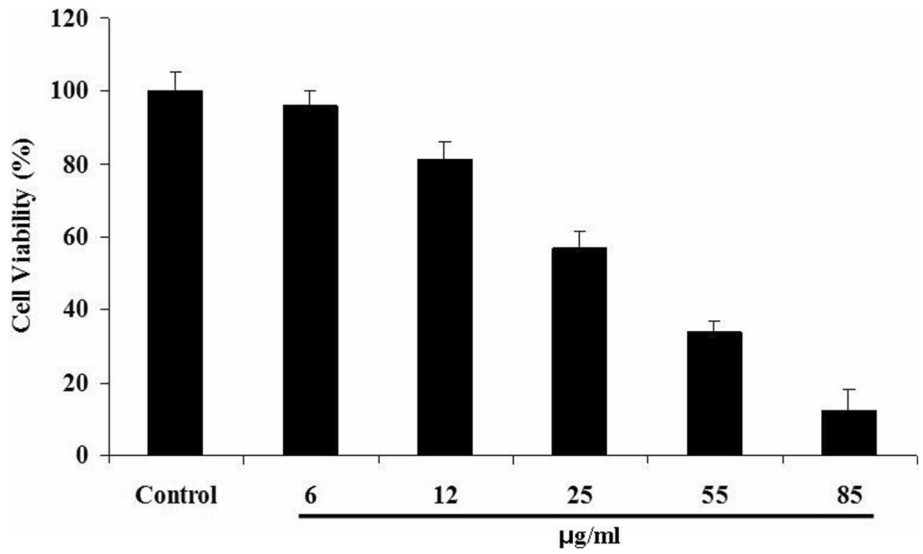


Fig. 3 Cell viability potential of 5-HMP with A-549

and ascorbic acid. Both 5-HMP and ascorbic acid neutralized the DPPH radicals with an IC_{50} value of 49.55 ± 0.75 μg/ml and 13.20 ± 1.25 μg/ml, respectively. 5-HMP acts as an antioxidant that acts by donating hydrogen atoms to obtain radicals with stable molecular structures that will stop the chain reaction by converting the unpaired electron to the paired electron.

In our study, both 5-HMP and ascorbic acid scavenged ABTS radicals with an IC_{50} value of 62.75 ± 1.25 μg/ml and 13.20 ± 1.25 μg/ml, respectively (Table 1, Fig. 2b). Our data evidenced that 5-HMP alkaloid isolated from *T. involucrata* can exhibit considerable antioxidant activity. We speculate that 5-HMP could be effective to prevent oxidative stress. We also studied the anti-cancer property of 5-HMP on human lung cancer cell line, A549.

Anti-lung cancer activity of 5-HMP

Chemoprevention or chemotherapy approach with least side effects is paramount interest of cancer drug discover researchers. To unravel anti-cancer role of 5-HMP, A549 cells were treated with different concentrations (6, 12, 25, 55, and 85 μg/ml) of 5HMP for 48 h viability of A549 cells reduced in increase concentration of the test drug (Fig. 3). IC_{50} value of 5HMP on A549 cells is 30.00 ± 0.55 μg/ml. The percentage inhibition of 5-HMP was assessed and showed that 5-HMP inhibits the cell growth (lung cancer cell line (A549) when increasing the concentration (Fig. 3).

Further, we studied the role of 5-HMP on A549 at different mitosis stages of the cells during proliferation. S and G2/M checkpoint blocks the entry into mitosis

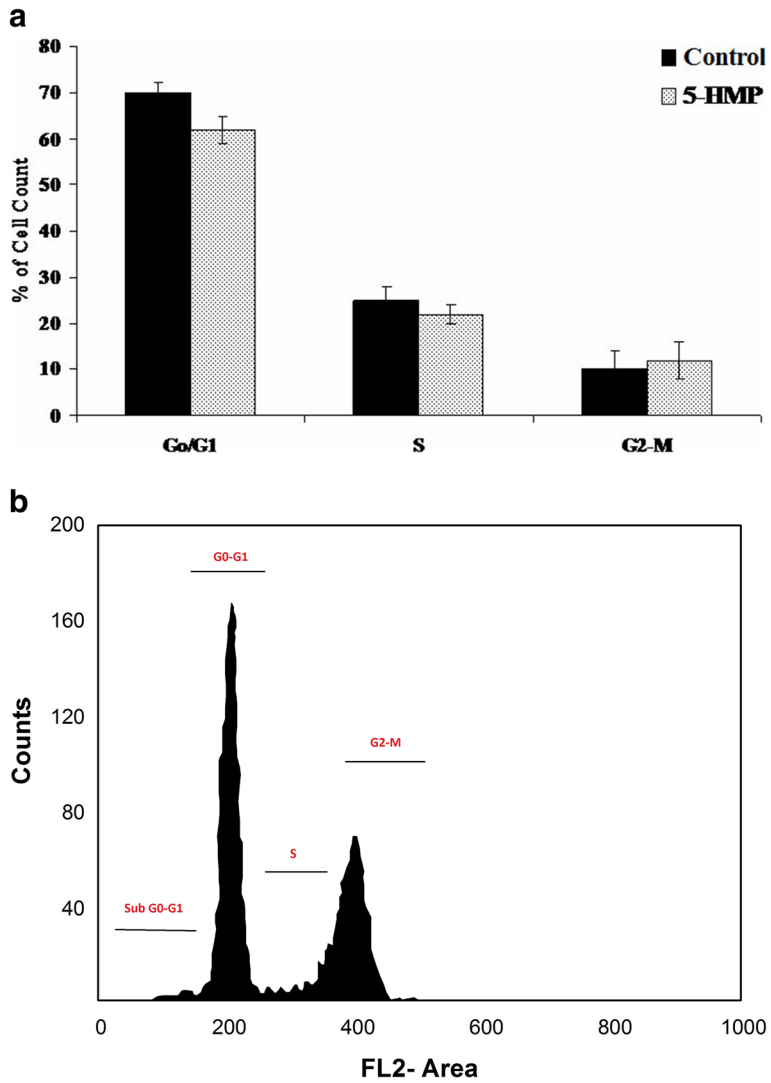


Fig. 4 a Effect of 5-HMP on A549 cell cycle**. b** Cell cycle distribution of 5-HMP (30 μg/ml) treated A549 cells

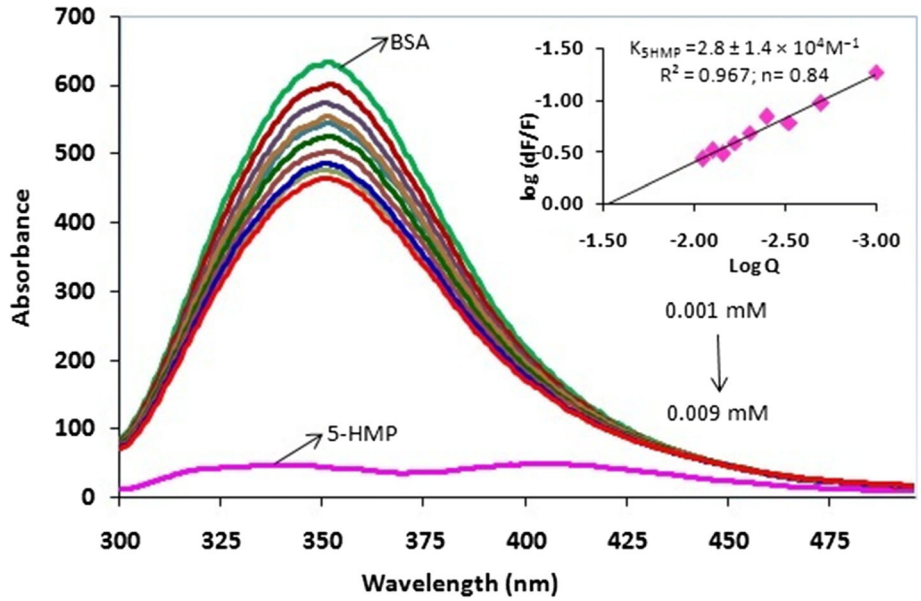


Fig. 5 Fluorescence quenching mechanism spectra of BSA-5-HMP emission under 25 °C, pH 7.4 physiological condition with constant concentration of BSA and increasing concentration of 5-HMP (0.001–0.009 mM), inside plot of log (dF/F) against log [Q]

when DNA is damaged [37]. A549 cells were extravagance with 5-HMP (30 μg/ml) for 24 h and analyzed for cell cycle arrest by flow cytometry. 5-HMP treatment showed dose-dependent cell cycle arrest in S and G2/M phase (Fig. 4a, b). Our data indicates that 5HMP halt DNA synthesis and subsequent mitosis in A549 cells. Phenolic compound treatment arrests S phase of cell cycle in prostate cancer cells and G2/M phase arrest in Hela cells [38, 39]. Further, Sanchez-Carranza et al. reported that natural compounds from *C. coriaria* induced the cell cycle arrest [39]. Extracts rich in phenolic compounds have shown S phase cell cycle arrest by inhibiting microtubule [40, 41]. These reports are in agreement with our results that 5-HMP

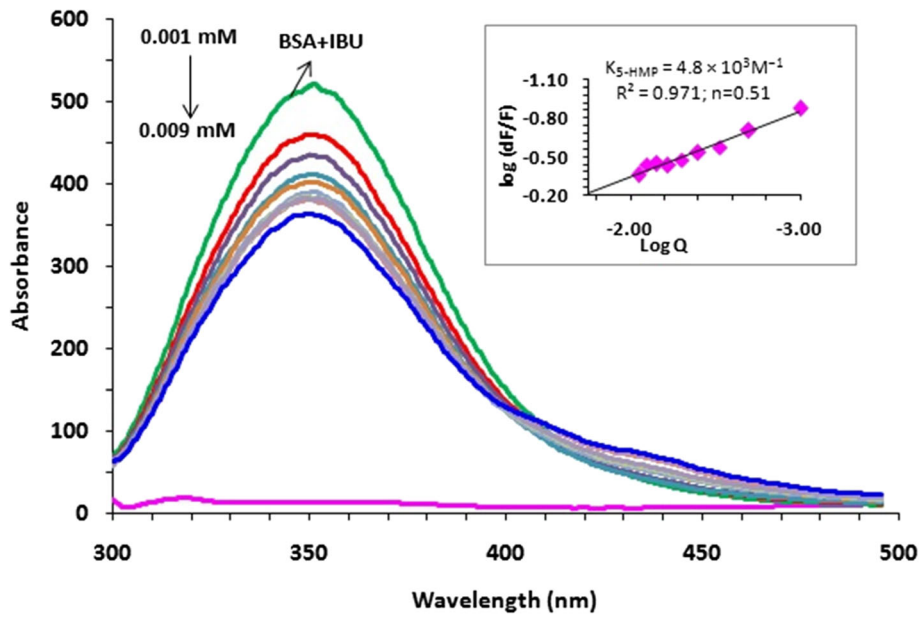


Fig. 6 Displacement experiment of BSA-5-HMP complexes with site-specific markers ibuprofen complex by 5-HMP (0.001 to 0.009 mM)

is phenolic compound arrest the cell cycle at S and G2/M phase in A549 cells.

Binding properties of 5-HMP on BSA

In vitro molecular-binding studies by fluorescence spectroscopy

The fluorescence quenching effects of 5-HMP on BSA was observed with their decreasing fluorescence intensity when increasing concentration of 5-HMP on constant concentration of BSA suggesting that it has interacted on BSA due to the decreasing fluorescence intensity at 350 nm with the physiological pH of 7.4. The micro environment changes such as the maximum absorbance was observed at 350 nm for BSA after addition of 5-HMP due to the florescence quenching mechanism on fluorescence emitting amino acid residue tyrosine, tryptophan, and phenylalanine. The binding constant of 5-HMP on BSA compared by in silico and found that the similar energy value to the fluorescence studies as − 4.7 K.cal/mole (Fig. 5) and the 5-HMP interacted with tryptophan residue − 275 (Fig. 9a, b). Here, we observe that static quenching mechanism is due to the formation of a ground-state complex between the fluorophore and quencher such as protein and ligand complex formation (Fig. 5).

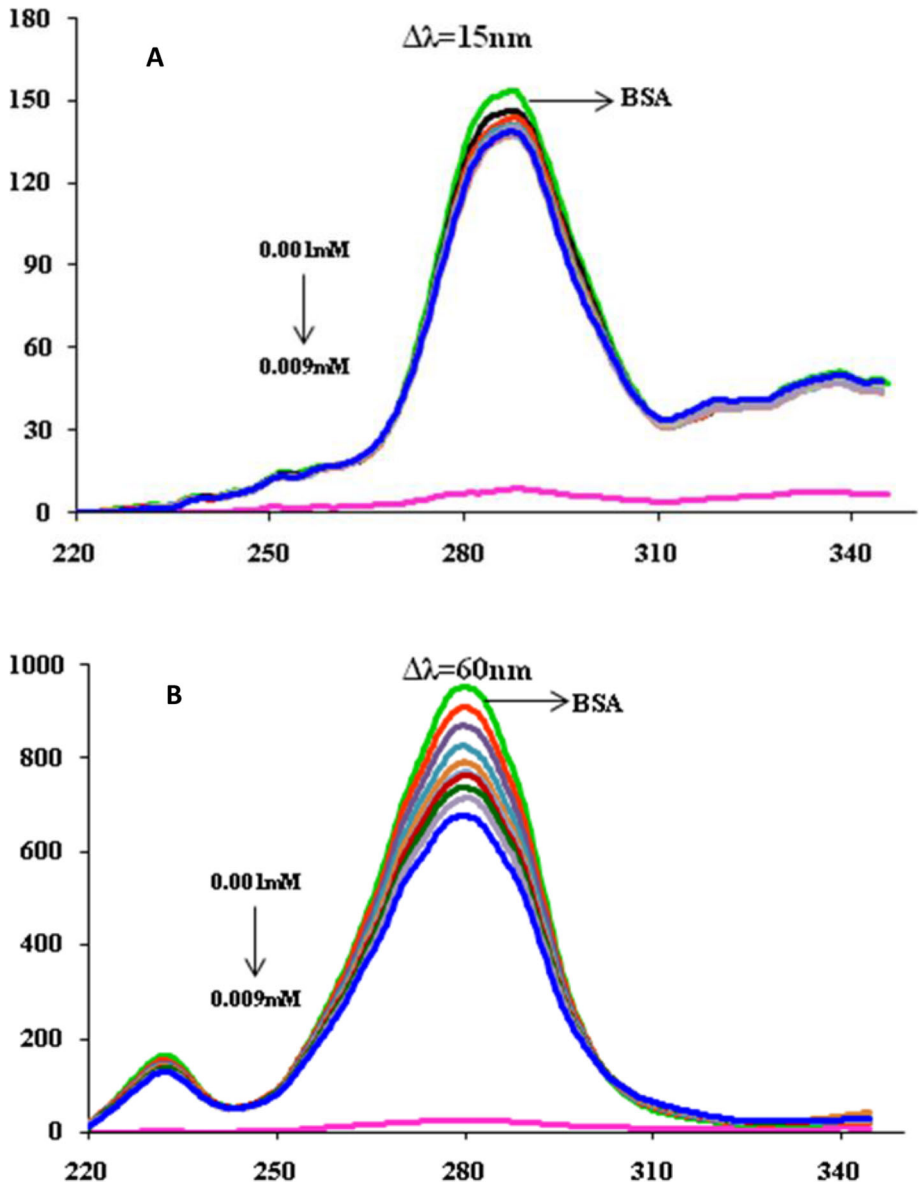


Fig. 7 The synchronous fluorescence emission spectra of BSA–5-HMP complex at different Δλ values. **a** Δλ15 for tyrosine residue and **b** Δλ60 for tryptophan residue

Displacement studies with site-specific markers

The study aims to find the location of 5-HMP on BSA using site-specific markers and find the binding affinity of lidocaine (4.9×10^3 M^{-1}), phenylbutazone ($6.9 \times 10^3 M^{-1}$), and lbuprofen ($4.8 \times 10^3 M^{-1}$) (Fig. 6). The data also showed that 5-HMP attaches to the region of IIA sub-domain of BSA due to ibuprofen being replaced better compared to the other site-specific markers and that ibuprofen contained lower binding values and binding energy [41]. The crystal structure of BSA contains the drug-binding site with hydrophobic packets in HA and IIIA subdomains with distinguished geometric conditions. It also has two tryptophan packets in IIA and IIIA subdomains with distinguished geometric conditions. It also has two tryptophan residues such as Trp 135 and Trp 212 [42], and the in silico molecular docking studies also revealed that 5-HMP has interacted with tryptophan on IIA subdomain of BSA.

Synchronous fluorescence studies of 5-HMP on BSA

The synchronous fluorescence spectral information can visualize the micro-environmental change in the BSA after addition of 5-HMP for the conformation of BSA-5-HMP complex with the optimum physiological condition (pH 7.4) due the occurrence of fluorescence emitting amino acid residue tyrosine, tryptophan, and phenylalanine on specific Δ values Δλ15, Δλ60, and Δλ90, respectively. The complex of BSA with 5-HMP were checked at Δλ15 for tyrosine residues and presented in Fig. 7. This shows that the fluorescence molecular quenching mechanism has observed on BSA-5-HMP complex due to the molecular interaction of 5-HMP with reduced absorbance on increasing concentration of 5-HMP [36, 43].

UV-Visible spectroscopic studies of 5-HMP on BSA

The UV-Visible spectra also showed good binding properties when the concentration of 5-HMP was increased at a fixed concentration of BSA (0.01, 0.025, 0.05, 0.075, and 0.1 mM, respectively) (Fig. 8). Figure 8 shows that millimolar concentration of 5-HMP has produced no maximum absorbance peak while adding on BSA gives a maximum absorption wavelength at 274 nm. The maximum absorption wavelength of BSA at 278 nm and the summation curve superposed by 5-HMP and BSA basically overlapped with the curve of the mixed solution, which indicates that BSA and 5-HMP did not form a new substance. Furthermore, when the concentration 5-HMP increased, there was an obvious change in the UV-VIS absorption spectra, which further provided evidence for a quenching mechanism [37].

In silico molecular docking studies of 5-HMP on BSA

The molecular docking studies used Autodock tool on windows platform. The energy minimized and structure optimized ligand (5-HMP) were docked on geometrically optimized BSA (PDB ID: 106) with 10 conformations. The energy values were compared with in vitro results and showed the energy value nearer to the in vitro experiments as – 4.7 K.cal/mole and the 5-HMP interacted with tryptophan residue-275 (Fig. 9a, b).

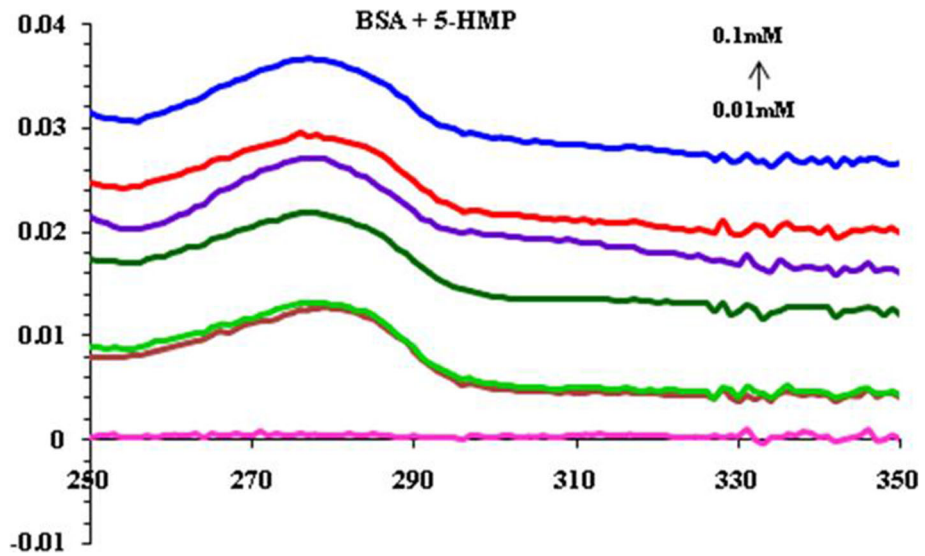


Fig. 8 UV-visible spectroscopic analysis of binding through absorbance between 270 nm to 290 nm on BSA with increase concentration of 5-HMP (0.01, 0.025, 0.05, 0.075, and 0.1 mM)

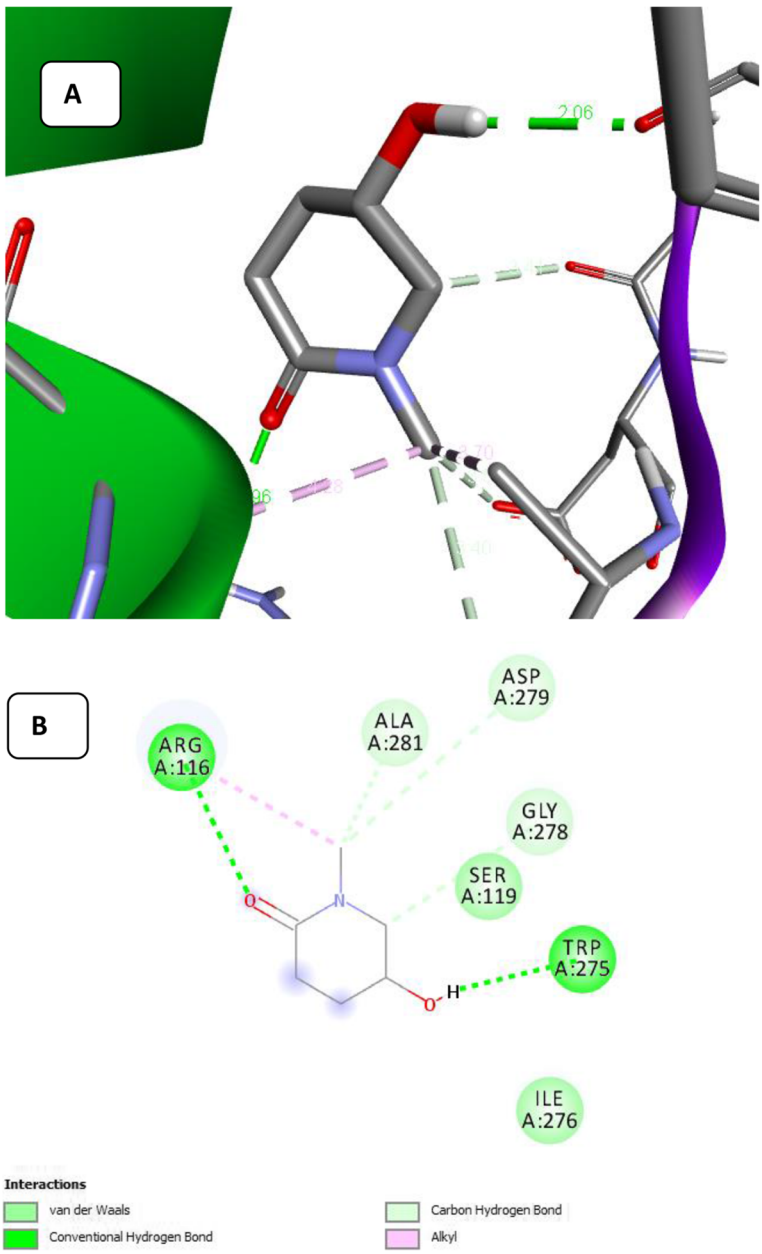


Fig. 9 Best docking conformation with the lowest binding energy. **a** An interaction on BSA and 5-HMP binding and **b** Ligplot showing hydrophobic interactions of HSA–5-HMP in the binding sites

Discussion

Reactive oxygen species (ROS) are produced by cellular metabolism in living cells. ROS have the potential to interact with cellular ingredients containing the deoxyribosyl backbone of DNA or DNA bases to generate strand breaks or damaged bases. ROS can also oxidize proteins or lipids afterward producing mediators that react with DNA by forming adducts. Many oxidative DNA damages are oxidative damage, and promutagenic which are suggested to participate in a significant function in the development of cancers [44]. Free radical scavengers play an immune role to alleviate the γ emission-induced oxidative damage in lung and breast cancer cell [45]. Therefore, in this study, we examined the antioxidant efficacy of 5-HMP using DPPH and ABTS radicals [46].

The observed cytotoxicity of 5-HMP could be due to induction of necrosis or apoptosis. Chemosensitivity of 5-HMP towards A549 cells is unclear. However, the observed anti-lung cancer activity of 5-HMP

could be due to the presence of alkaloid unit with hydroxyl group of the molecule on its structure. It is well reported that hydroxylated alkaloid compounds are potent anti-cancer agents, and they restrict the proliferation of breast cancer cells (MCF-7) [47, 48]. Hydroxyl groups can intercalate with DNA and execute irreversible DNA damage which in turn leads to nuclear fragmentation. Further phenolic compounds are well reported as an inducer of apoptosis in HEK293T and K562 cells [49, 50].

Similar binding was reported with various phytomolecules such as resveratrol and genistein on BSA as 2.52 ± 0.5 × 10^4M^{-1} and 1.26 ± 0.3 × 10^4M^{-1} [51]. The gradual decrease was observed when the concentration of 5-HMP was increased at a fixed concentration of BSA and the same was reported with tetraphenyl porphyrin on BSA [52]. Also recently reported is the Azoimine quinoline derivatives that show good binding affinity on BSA and DNA [53]. The nearer binding constant for sulfacetamide sodium on BSA was reported by Naik et al. 2010 [54] as $K_{Sulfacetamide\ Sodium}$ = 2.0072 × 10^4M^{-1} with temperature dependent. Decrease of the quantum defer of fluorescence from a fluorophore induced by small molecular exchanges and their mode of actions [55]. The same type of interactions was observed when resveratrol and genistein reacted on Trp 212 and Trp 134 in silico [51].

Conclusion

5-HMP is naturally occurring piperidine alkaloid found in *T. involucrata.* It acts as a potent free radical scavenger and an anti-lung cancer agent. 5-HMP restricts the growth of A549 cells by arresting S and G2/M phases of cell cycle. Furthermore, our in vitro studies revealed that 5-HMP has good binding constant on BSA, and that it has interacted on tryptophan residue of the protein which could increase its bioavailability and therapeutic efficacy. Further, in vivo pre-clinical studies are needed to confirm the therapeutic potential of the 5-HMP.

Abbreviations

5-HMP: 5–hydroxyl–1–methylpiperidin–2-one; BSA: Bovine serum albumin; ROS: Reactive oxygen species; PDB: Protein Data Bank; DPPH: 2,2-diphenyl-1-picrylhydrazyl

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Authors' contributions

S.A.Y has designed and executed this research work and written the manuscript. L.H.F and H.B had modified the manuscript. R.S has calculated the binding studies. L.K.S has helped to perform the cytotoxicity studies. Overall, all the authors have contributed to make a manuscript. Finally all authors have read and approved the manuscript.

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Availability of data and materials

All data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Biotechnology, Karpagam Academy of Higher Education, Eachanari - Post, Coimbatore 641021, Tamil Nadu, India. [2]Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Salalah, Oman. [3]Research Centre, Dhofar University, Salalah, Oman. [4]Department of Plant Science, University of Hyderabad, Hyderabad, Telangana, India. [5]Department of Biotechnology, Kongunadu College of Arts and Science, Coimbatore, Tamil Nadu, India. [6]School of Pharmacy and Pharmaceutical Science, Ulster University, ColeraineCounty Londonderry, BT52 1SA, Northern Ireland, UK.

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References

1. American Cancer Society (2018) Cancer facts & figures, pp 1–71
2. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, Bray F (2020) Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Global Health *8*(2):e191–e203. https://doi.org/10.1016/S2214-109X(19)30482-6
3. Huy LAP, He H, Huy CP (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci. 4(2):89–96
4. Zablocka-Slowinska K, Porębska I, Gołecki M, Kosacka M, Pawełczyk K, Pawlik-Sobecka L et al (2016) Total antioxidant status in lung cancer is associated with levels of endogenous antioxidants and disease stage rather than lifestyle factors – preliminary study. ContempOncol (Pozn). 20(4):302–307
5. Von Pawel J, Schiller JH, Shepherd FA, Fields SZ, Kleisbauer JP, Chrysson NG et al (1999) Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. J Clin Oncol. 17(2):658–667. https://doi.org/10.1200/JCO.1999.17.2.658
6. Deba F, Xuan TD, Yasuda M, Tawata S (2008) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidenspilosa*Linn. var. Radiata. Food Control. 19(4):346–352. https://doi.org/10.1016/j.foodcont.2007.04.011
7. Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian medicinal plants, vol 1. Council of Scientific and Industrial Research, New Delhi, pp 1–197
8. PerumalSamy R, Ignacimuthu S, Sen A (1998) Screening of thirty-four Indian medicinal plants for antibacterial properties. J Ethnopharmacol 62(2):173–182. https://doi.org/10.1016/S0378-8741(98)00057-9
9. Palani S, Nirmal Kumar S, Gokulan R, Rajalingam D, Senthil Kumar B (2009) Evaluation of Nephroprotective and antioxidant potential of *Tragia involucrate*. Drug Invent Today 1*(1)*:*55–60*
10. Joshi C, Gopal M, Byregowda SM (2011) Cytotoxic activity of *Tragia involucrate*. Linn. Extracts. Am-Eurasian J Toxicol Sci 3(2):67–69
11. Farook M, Atlee WC (2011) Antioxidant potential of *Tragiainvolucrata*linn on streptozotocin induced oxidative stress in rats s. Int J Pharmaceut Sci Res 2(6):1530–1536
12. Vinodhini V, Himaja M, Saraswathi VS, Poppy D (2015) In vitro anti diabetic activity of *Tragiainvolucrata* Linn leaf extracts. Int J Res Ayurveda Pharm 6(1):1–3. https://doi.org/10.7897/2277-4343.0611
13. Abdul Rahman S, Anazi A, Anwar MJ, Ahmad MA (2015) Hepatoprotective and antioxidant activity of *Tragia involucrata* root extracts against CCl4 induced hepatotoxicity in rats. Der Pharmacia Lettre 7(5):146–152

14. Yadav SA, Ramalingam S, Raj AJ, Subban R (2015) Antihistamine from *Tragiainvolucrata* L. leaves. J Complement Integr Med. 12(3):217–226
15. Hu YJ, Liu Y, Shen XS, Fang XY, Qu SS (2005) Studies on the interaction between 1-hexylcarbamoyl-5-fluorouracil and bovine serum albumin. J MolStruct 738(1-3):143–147. https://doi.org/10.1016/j.molstruc.2004.11.062
16. Kamat BP, Seetharamappa J (2005a) *In vitro* study on the interaction of mechanism of tricyclic compounds with bovine serum albumin. J ChemSci 117:649–655
17. Kamat BP (2005b) Spectroscopic investigations on the interaction of bovine serum albumine with amoxicillin and cloxacillin. J Photosci 12:11–15
18. Kamat BP (2005c) Study of the interaction between fluoroquinolones and bovine serum albumin. J Pharm Biomed Anal 39(5):1046–1050. https://doi.org/10.1016/j.jpba.2005.05.013
19. Cater DC, Ho JX (1994) Structure and ligand binding properties of human serum albumin. Adv Protein Chem 45:153–203. https://doi.org/10.1016/S0065-3233(08)60640-3
20. Olson RE, Christ DD (1996) Plasma protein binding of drugs. Ann Rep Med Chem 31:327–337
21. Wanwimolruk S, Denton JR (1992) Plasma protein binding of quinine: binding to human serum albumin, α1-acid glycoprotein and plasma from patients with malaria. J Pharm Pharmacol 44(10):806–811. https://doi.org/10.1111/j.2042-7158.1992.tb03210.x
22. Benet LZ, Kroetz D, Sheiner L, Hardman J, Limbird L (1996) Pharmacokinetics: the dynamics of drug absorption, distribution, metabolism, and elimination. Goodman Gilman's Pharmacol Basis Therapeut 3:27.
23. He XM, Carter DC (1992) Atomic structure and chemistry of human serum albumin. Nature 358(6383):209–215. https://doi.org/10.1038/358209a0
24. Carlo B, Giorggio A, Gloria UB (1995) J Pharm Biomed Anal 13:1087–1093
25. Janna O, Dagmar S, Wolfgang L (1996) J Chromatogr B 682:349–357
26. Longworth JW (1971) In: Steiner RF, Weinryb I (eds) Excited states of proteins and nucleic acids. Plenum Press, New York, pp 433–434
27. Luigi M, Francesca P, Silvia G (2002) Bioorg Med Chem 10:3425–3430
28. Sułkowska A (2002) Interaction of drugs with bovine and human serum albumin. J Mol Struct 614(1-3):227–232. https://doi.org/10.1016/S0022-2860(02)00256-9
29. Liu JQ, Tian JN, Tian X, Hu ZD (2004) Interaction of isofraxidin with human serum albumin. Bioorg Med Chem 12:469–474
30. Yun BS, Qian SD, Yuan T, Xin Z (2005) Molecular spectroscopic study on the interaction of tetracyclines with serum albumins. Spectrochim Acta A 61(4):629–636
31. Mondal SK, Chakraborty G, Gupta M, Mazumder UK (2006) *In vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. IJEB 44(01):39–44
32. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free RadicBiol Med 26(9-10):1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3
33. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1-2):55–63
34. Nelson SS, Yadav SA, Surendren LK (2019) Evaluation of in vitro anticancer potential in *Punica granatum, Psidium guajava,* and *Vitis vinifera* seed extracts. Int J Res Pharmaceut Sci. 10(1):165–169
35. Yadav SA, Yeggoni DP, Devadasu E, Subramanyam R (2018) Molecular binding mechanism of 5-hydroxy-1-methylpiperidin-2-one with human serum albumin. J Biomol Struct Dyn 36(3):810–817
36. Huazhen Y, Bin Q, Zhenyu L, Guonan C (2011) Fluorescence spectrometric study on the interaction of tamibarotene with bovine serum albumin. Luminescence 26:336–341
37. Taylor WR, Stark GR (2001) Regulation of the G2/M transition by p53. Oncogene 20(15):1803–1815
38. Agarwal C, Tyagi A, Agarwal R (2006) Gallic acid causes inactivating phosphorylation of cdc25A/cdc25C-cdc2 via ATM-Chk2 activation, leading to cell cycle arrest, and induces apoptosis in human prostate carcinoma DU145 cells. Mol Cancer Ther 5(12):3294–3302
39. Tan S, Guan X, Grün C, Zhou Z, Schepers U, Nick P (2015) Gallic acid induces mitotic catastrophe and inhibits centrosomal clustering in HeLa cells. Toxicol In Vitro 30(1 Pt B):506–513
40. Tagne RS, Telefo BP, Nyemb JN, Yemele DM, Njina SN, Goka SM et al (2014) Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. Asian Pac J Trop Med. 7:S442–S447. https://doi.org/10.1016/S1995-7645(14)60272-8
41. Li J, Yao P (2009) Self-assembly of ibuprofen and bovine serum albumin–dextran conjugates leading to effective loading of the drug. Langmuir 25(11):6385–6391. https://doi.org/10.1021/la804288u
42. Lakowicz JR (1983) Principles of fluorescence spectroscopy. Plenum, New York, London. https://doi.org/10.1007/978-1-4615-7658-7
43. Hu YJ, Liu Y, Wang JB (2004) Study of the interaction between monoammoniumglycyrrhizinate and bovine serum albumin. J Pharm Biomed Anal **36**(4):915–919. https://doi.org/10.1016/j.jpba.2004.08.021
44. Dreher D, Junod AF (1996) Role of oxygen free radicals in cancer development. Eur J Cancer 32A(1):30–38. https://doi.org/10.1016/0959-8049(95)00531-5
45. Petkovic VD, Keta OD, Vidosavljevic MZ, Incerti S, RisticFira AM, Petrovic IM (2018) Biological outcomes of γ-radiation Induced DNA damages in breast and lung cancer cells pretreated with free radical scavengers. Int J Radiat Biol. 19:1–34
46. Miller NJ, Rice-Evans CA (1996) Spectrophotometric determination of antioxidant activity. Redox Rep *2*(3):161–171. https://doi.org/10.1080/13510002.1996.11747044
47. Kahl R, Kappus H (1993) Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z Lebensm-Unters Forsch. 196(4):329–338. https://doi.org/10.1007/BF01197931
48. Fernandes I, Faria A, Azevedo J, Soares S, Calhau CAO, Freitas VD et al (2010) Influence of anthocyanins, derivative pigments and other catechol and pyrogallol-type phenolics on breast cancer cell proliferation. J Agric Food Chem. 58(6):3785–3792
49. Mitsuhashi S, Saito A, Nakajima N, Shima H, Ubukata M (2008) Pyrogallol structure in polyphenols is involved in apoptosis induction on HEK293T and K562 Cells. Molecules 13(12):2998–3006. https://doi.org/10.3390/molecules13122998
50. Snchez-Carranza JN, Alvarez L, Bahena SM, Vidal ES, Cuevas V, Jimenez EW et al (2017) Phenolic compounds isolated from Caesalpiniacoriaria induce S and G2/M phase cell cycle arrest differentially and trigger cell death by interfering with microtubule dynamics in cancer cell lines. Molecules 22(4):2–14
51. Bourassa P, Kanakis CD, Tarantilis P, Pollissiou MG, Tajmir-Riahi HA (2010) Resveratrol, genistein, and curcumin bind bovine serum albumin. J Phys Chem B 114(9):3348–3354. https://doi.org/10.1021/jp9115996
52. Tian J, Liu X, Zhao Y, Zhao S (2007) Studies on the interaction between tetraphenylporphyrin compounds and bovine serum albumin. Luminescence 22(5):446–454. https://doi.org/10.1002/bio.983
53. Douadi K, Chafaa S, Douadi T, Al-Noaimi M, Kaabi I (2020) Azoimine quinoline derivatives: Synthesis, classical and electrochemical evaluation of antioxidant, anti-inflammatory, antimicrobial activities and the DNA/BSA binding. J Mol Struct 1217:128305. https://doi.org/10.1016/j.molstruc.2020.128305
54. Naik PN, Chimatadar SA, Nandibewoor ST (2010) Pharmacokinetic study on the mechanism of interaction of sulfacetamide sodium with bovine serum albumin: a spectroscopic method. Biopharmaceut Drug Dispos. 31(2-3):120–128. https://doi.org/10.1002/bdd.696
55. Bhattacharyya M, Chaudhuri U, Poddar RK (1990) Evidence for cooperative binding of CPZ with hemoglobin. Biochem Biophys Res Commun. 167(3):1146–1153. https://doi.org/10.1016/0006-291X(90)90643-2

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