

Pharmacological and Pharmacokinetic Studies of Hardwickia binata Roxb. Leaf Extract In Insilico and Invitro Models

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Abstract

To find new sources of antimicrobial and antioxidant agents, methanol extracts of *Hardwickia binata* Roxb were evaluated systematically. To the present investigation for potential antibacterial and antioxidant phytochemicals in this study and also ADMET and molecular docking for CADD studies. Our results the polyphenol content (total phenol, total flavonoid) and antioxidant capacity of methanol extracts were examined. Identify skin cancer active sites from *Hardwickia binata* bioactive compounds using GCMS and molecular docking, network pharmacology. The free radical scavenging activities of the methanol extracts also were highest, with the antioxidant activity becoming significantly greater. Furthermore, *in vitro* antibacterial experiments against phytopathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aureginosa* validated LE's antimicrobial efficacy. Compared to the other pathogens *Enterococcus faecalis* have high zone of inhibition (14 ± 0.54). Chemical composition of crude drug extracts was also performed by identify functional groups such as N–H, C–H, OH, and C = O in the methanol extract. GC-MS investigation revealed the existence of 30 bioactive chemicals and 7 active molecules were identify with ADMET and docking in skin cancer proteins (1P7K and 5OTE) among the phytocompounds, Quinoline, 1,2-dihydro-2,2,4-trimethyl (-4.2 kcal/mol; -4.6 kcal/mol), Hexadecanoic acid, methyl ester (-6. kcal/mol; -6.2 kcal/mol) and 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative (-8.11 kcal/mol; -7.50 kcal/mol) *Hardwickia binata* is the best compound for the human skin cancer possessed higher binding energy. Our results indicate that the plants can provide sources of natural compounds and the docking studies were carried out in order to predict the most probable kind of interaction, binding affinities, and orientations of the docked ligands at the target protein's active site.

Introduction

Natural products an important role in the treatment and prevention of human ailments all over the world. Medicines have come from a variety of sources, including plants, microorganisms, marine organisms, vertebrates and invertebrates, but medicinal herbs are a major source of both synthetic and herbal drugs. Medicinal herbs are sold as raw medications, extracts and tinctures in the commercial market. Herbal products are becoming increasingly popular [1]. Although some herbal medications have a lot of promise and are extensively used, many of them haven't been studied and their use is also not regulated. This limits our knowledge of their potential side effects, making it more difficult to identify the safest and most effective therapies and encourage their rational use [2].

Secondary metabolites have therapeutic potential for disorders caused by oxidative stress. All living organisms that have an aerobic mode of respiration produce reactive oxygen species (ROS). These ROS are found in cell metabolism, including intercellular signalling, cell defence responses, phagocytosis, and energy production. Antioxidants keep these reactive species in balance [3]. Phytocompounds have considerable effects on a range of pharmaceutical products [4].

Antimicrobial resistance (AMR) is a global challenge which has reduced our ability to effectively treat infectious diseases in 21st century [5]. While numerous factors contribute to the emergence of AMR, inappropriate antibiotic prescriptions and their use in agriculture are prominent [6]. AMR-related mortality have increased to roughly 750,000 per year, with estimates of up to 10 million by 2050 [7]. Infections due to pathogenic microorganisms cause a severe concern to human health. Increasing cases of drug resistance, unwanted side effects of existing antibiotics and the reappearance of earlier known infections have demanded the need for new, safe and effective antimicrobial agents [8]. Among the fungi, metabolites produced by the *Trichoderma* species are known to have anticancer activity. Skin cancer is growing as a dreadful human disease as compared to other cancers. However, potent drugs to cure skin cancer are increasingly necessary [9]. The search for anti-cancer compounds from plant sources with using the development and discovery of the various natural compounds like vinca alkaloids, vinblastine, vincristine and cytotoxic podophyllotoxins [10]. In drug development, virtual screening like drug-likeness and ADMET analysis are computational methods to find compounds that are likely to exhibit physiological activity [11].

Hardwickia binata is a member of the Fabaceae family, which is also known as "Anjan." It is a huge deciduous ornamental tree that grows to a height of 25–300 feet and is dark grey. It is monotypic genus flowering plant synonyms are *Hardwickia trapeziformis* R. Grah. *Harongana madagascariensis* chois. This genus is represented by single species *Hardwickia binata*. It is medicinally and economically important plant which yield hard, durable quality of timber and used as firewood as charcoal [12]. Bark is rich in tannins used as gums and resins. The gum of this tree used to treat gonorrhoea. A decoction made from bark is used to treat piles, toxemia, skin disease and dysentery [13]. It is used for folklore medicine for various ailments diarrhoea, worms, indigestion and leprosy and produce an appetizer. The leaves of *Hardwickia binata* are used as fodder and manure [14].

This study was conducted to determine the phytochemical composition (total phenolic and flavonoid contents) in methanol extract of *Hardwickia binata*, as well as to inspect the antioxidant activity, antibacterial activity and to identify bioactive compounds using *in-silico* analysis and to understand the pharmaceutical potential of the isolated compounds via network pharmacology and molecular docking.

Results And Discussion

Percentage of yield

The extraction of *Hardwickia binata* leaf in organic solvent showed good yield compared to others like ethyl acetate and hexane. Results showed the significant difference in yield using different solvents. Among solvents tested, methanol showed higher extraction of Yield (3.1%) followed by ethyl acetate (2.7%) and Hexane (2.4%), indicating the extraction efficiency favors the high polar solvents and methanol extracts showed high polyphenol and terpenoid content [15]. Similar results were finding in *Knoxia sumetrensis* methanolic extract of yield 3.1% [16] and methanol extract of *Citrullus colocynthis* yield 3.20% [17]. It is possible that the high yield in aqueous media is owing to the fact that most phytochemicals have electronegative functional groups, making the compound hydrophilic in nature [18].

Qualitative analysis of *H.binata*

The phytochemical screening of *Hardwickia binata* was analysed in methanol leaf extract. (Supplementary file, Table 1) Results shows the presence and absence of secondary metabolites. Alkaloids, phenol, Tannin, Flavonoids, Steroids and glycosides, Carbohydrate and terpenoids were present in methanolic leaf extract of *H. binata* and proteins are absent.

Quantitative analysis

Estimation of total phenolic content

The total phenolic content was measured using different concentration of Gallic acid equivalents in milligram per gram (mg GAE/g) of dry weight (DW). Our results demonstrated total phenolic content of methanol extract of *Hardwickia binata* ($Y = 0.004x + 0.018$, $R^2 = 0.9995$). Similar results were finding in methanol extract of *Ficus carica* ($R^2 = 0.953$) and *Olea europaea* ($R^2 = 0.973$) [19]. Phenol and flavonoids are correlated to antioxidants with the ability to break chains [20]. Phenolic compounds exhibit good antioxidant activity, because the plant extract contains the hydroxyl group responsible for facilitating free radical scavenging [21]. Phenolics can serve as antioxidants in a range of methods. Hydroxyl groups in phenolics are good hydrogen donors: hydrogen-donating antioxidants may interact with reactive oxygen and nitrogen species in a termination reaction, breaking the cycle of radical formation [22].

Total flavonoid content

Total flavonoid content of *H. binata* differs according to different concentration of sample. The results are expressed as mg/g of rutin equivalent. Total content of flavonoid was expressed in methanol extract of *Hardwickia binata* leaf ($Y = 0.059x + 0.019$) $R^2 = 0.996$. Equivalent in previously reported the flavonoid content of *Ficus carica* ($R^2 = 0.922$) and *Olea europaea* ($R^2 = 0.934$) [19]. Flavonoids have strong free radical scavenging of free radicals, chelation of metal ions and oxidizing molecules to implicate various diseases [23].

Functional group identification of Methanol leaf extract *Hardwickia binata*

The FTIR spectrum of *Hardwickia binata* methanol leaf extract and ethyl acetate extracts were analysed from 500 cm⁻¹ to 4000 cm⁻¹ (fig.1) and the intensity of peak, group and character (table 1). The dominant stretching aliphatic primary amine (N-H) group's intense peak 3370.50 cm⁻¹. Stretching alkene group (C-H) is responsible for the 2916.70 cm⁻¹ peak and strong stretching group of carbon dioxide (O=C=O) is represented by the band at 2362.94 cm⁻¹. The peak at 667.70 cm⁻¹ report (C=C) strong alkene group. Strong primary alcohol group indicate the intense peak at 1053.53 cm⁻¹. Similar results are reported in methanolic leaf extract of *Coriandrum sativum* have functional group such as amines (N-H), alkene (C=C) and primary alcohol (C-O) was observed [24]. In other studies, functional group such as N-H (amine), C-H (alkene), C=O (Primary alcohol) were observed from *Schefflera vinnosa* leaf extract [25].

Phytochemicals identification of MeOH - LE *Hardwickia binata*

Phytochemicals constitutions of methanol leaf extracts were identified by using GC-MS. Methanol extract show 30 compounds in chromatogram, which were identified based on retention time between 3 to 28 min (fig. 2). The name, molecular structure, molecular formula and biological activities of the identified compounds are given in Table 2 (supplementary file). Among these 30 compounds; six biomolecules were identified as Hydrazinecarbothiomide, Undecane, Mome Inositol, N-Hexadecanoic Acid, 1-Hexadecanol, Acetate, 1,3-Benzenedicarboxylic Acid, Bis (2-Ethylhexyl) Ester. These reported phytomolecules have anticancer [26] anti-allergic, anti-inflammatory [27], anti-allopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic [28], antioxidant, nematocide, anti-androgenic, Haemolytic, Pesticide [29]. In similar results were finding in *Paracoccus pantotrophus* contains tetratriacontane, heneicosane, hexadecanoic acid, methyl ester, eicosane, tetracosane, 1-heptacosanal [30]. The bioactive compound of ethanol leaf extract of *Pergulaia daemia* was shown tetracosamethyl-cyclododecasiloxae, 1,3 benzenedicarboxylic acid, bis (2-ethylhexyl) ester [31]. Other results were finding in endangered plant *Ceropegia bulbosa* chloroform extract of whole plant shown Eicosane, hexatriacontane, octadecanoic acid [32].

Biological applications

Antibacterial activity

The *H. binata* of Methanol leaf extracts of antibacterial activity was carried out in disc diffusion method using gram-positive and gram negative bacteria (fig. 3). In higher zone of inhibition was observed in gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) compared to negative bacteria (*Escherichia coli* and *Pseudomonas aureginosa*). In comparison of negative bacteria, positive bacteria have higher zone inhibition (*Enterococcus faecalis* (14 ± 0.54 mm) and *staphylococcus aureus* (12 ± 0.63 mm) (Table 2). In comparison of positive bacteria, negative bacterial strains have less susceptible action because the presence of thin layer of peptidoglycan. In positive bacterial cell wall rich in peptidoglycan and polysaccharides. This structural difference may cause the low antibacterial activity in negative bacteria (*Escherichia coli* and *Pseudomonas aureginosa*) and higher activity in positive bacterial strains (*Enterococcus faecalis* and *staphylococcus aureus*) [33]. In similar results were finding in ethanolic extract of *Psidium quayaquilense* antibacterial activity against *staphylococcus aureus* (14 mm) [34] and *Enterococcus faecalis* (17 mm) using methanolic callus extract of *Saraca asoca* [35]. Other results were finding in antibacterial activity against *Pseudomonas aureginosa* (11 mm) in *Pongamia pinnata* [36] and cold water extract of *Jasminum officinale* antibacterial activity against *Escherichia coli* (7 mm) [37].

Antioxidant activity

The DPPH technique works on the basis of reducing DPPH in the presence of a hydrogen donating antioxidant [38]. The ability of extracts to donate hydrogen reduces the colour of DPPH. DPPH is one of the chemicals that contains a proton free radical with a distinct absorption that is greatly reduced when exposed to proton radical scavengers [39]. Scavenging activity of *H. binata* was carried out in DPPH assays. The free radical inhibition were increasing concentration (50, 100, 150, 200, 250 µg/ml) in a dose dependent manner of MeOH leaf extracts and standard were shown in fig 4. The highest free radical inhibition

percentage was observed in maximum concentration (250 µg/ml) in 80% of inhibition. The studies clearly reveal that natural antioxidants obtained from natural have high radical scavenging activities, which are crucial to preventing the harmful effects of free radicals in diabetes [40]. Antioxidant activity using DPPH of *H. binata* IC₅₀ (306.85 ± 3.35 µg/mL). In similar results were finding DPPH activity in *Chaptalia nutans* methanolic leaf extract (345.41 ± 5.35 µg/mL) [41]. Another report were finding in *Alstonia angustifolia* (IC₅₀ = 384.77 µg/mL) ethanolic leaf extract [42].

ADMET

Many potential therapeutic drugs fail to progress to clinical trials due to negative ADMET (absorption, distribution, metabolism, elimination, and toxicity) parameters. This research includes quantitative measurements of drug-like properties, such as pKa, absorption, solubility, bioavailability, BBB penetration and so on [43]. The Lipinski, Veber, and Igan rules are based on a review of human medication ADME properties. These principles are incredibly beneficial for generating drugs based on the 2D structure of tiny molecules as well as the bioavailability of those molecules. All of the isolated compounds match Lipinski's rule of five with zero violations, according to ADME's computations [44]. We also evaluate other factor like, number of rotatable bonds (n-ROTB), topological polar surface area (TPSA), octanol-water coefficient (logP), HBD and HBA [45]. All of the compounds' Kp values are between -4.26 and -5.47 cm/s indicating limited skin permeability. They exhibit ideal lipophilicity, according to the predicted logP values (ranging from 2.43 to 4.37). Among 27 compounds, 13 compounds exhibit high gastrointestinal (GI) absorption and 5 compounds exhibit blood-brain barrier (BBB) permeability showed in table 3, there are Quinoline, 1,2-dihydro-2,2,4-trimethyl, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 4,4'-(p-Phenylene) diisopropylidene diphenol, 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative, that according ADME prediction parameters. All of the drugs are CYP1A2, CYP2C19, and CYP2C9 inhibitors, as per the CYP interaction results (table 4). One of most essential enzymes involved in the drug metabolism is CYP2D6 [46]. Compounds 3, 4, 23 and 26 were identified as probable CYP2D6 inhibitors, while other compounds were identified as non-inhibitors.

Molecular docking

The molecular docking is used to explore the molecular interaction of ligand and target. A careful examination of molecular docking interactions could aid in the development of ligands with the properties required for efficient binding to the target receptor, resulting in the accomplishment of organizational goals pharmacological and therapeutic Agents. Table.5 displays the binding energy, number of hydrogen bond contacts, bond length and the amino acids involved in the interactions. The result showed that most of the ligands (1P7Kand 5OTE) established amino acid interaction frequently with AQE: 501, ALA: 86 and PRO: 44, TYR: 36, PHE: 98. All hydrogen bond interaction showed hydrogen bond length below 2.6Å. The best docking (binding free energy) scores for all active bio compound were found in the range with 1P7K (Quinoline, 1,2-dihydro-2,2,4-trimethyl -4.6 / amino acid residues PRO:44 (4.11), TYR:36 (4.56) TYR:87 (4.37, 4.87) PHE:98 (4.76, 3.81), Hexadecanoic acid, methyl ester -6.2 kcal/mol/ residues of amino acids interaction LEU:46 (2.18), TYR:36 (4.44, 4.32) PRO:44 (5.02, 5.12), PHE:98 (3.63, 3.89, 4.30, 4.44), TYR:87 (3.99), and 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative = -7.50/ amino acid residues PHE:98 (2.20, 5.29, 4.88, 4.16) TYR:87 (4.16), TYR:36 (4.90) PRO:44 (4.89)) (fig. 5-7) and 5OTE (Quinoline, 1,2-dihydro-2,2,4-trimethyl = -4.2/ residues of amino acid interaction LYS:109 (4.82) VAL:35 (3.89) ASP:32 (2.21) CYS:36 (5.14), Hexadecanoic acid, methyl ester = -6.6/ interaction of amino acid residues SER:221 (2.82), THR:118 (3.70), VAL:90 (5.11) AQE:501 (4.74), PHE:87 (5.15), ALA:86 (4.71), LYS:105 (4.63) and 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative = -8.11/ amino acid interaction THR:239 (2.26) LYS:202 (2.29), ASP:200 (3.66) ASP:218 (4.85) ALA:86 (4.82), AQE:501 (3.91, 2.10) ASP:201 (2.00)). (fig. 8-10).

Network Analysis of Protein-Protein Interactions (PPI)

Thirty-one primary hub nodes were selected and inserted into the STRING database, creating a link between two different nodes proteins/genes). The PPI network (44 edges and 21 nodes) was constructed (Figure 11) and may be important in the pharmaceutical effect phase of oncology. GTF31, RASL12, FCHO2 and CMIP were the only three targets that did not connect with one another. A rating of 0.883 or above indicates a high level of trust. UBXN2B, UBXN7, AMN and TRAF were two independent nodes with high degrees of trust.

Network Pharmacology

Molecular Analysis

At the statistical level, the *p*-value was 3.92e-05 < 0.65. AT was mostly associated with the MF 34 and led to biological quality control, prostate gland growth, control of prostate epithelial cell proliferation found by transmission, and gene-set enrichment analysis knowledge along with a gene analysis set improvement for these 21 genes. GTF31, RASL12, FCHO2 and CMIP proteins have common biological functions as well molecular functions (specifically the characteristics of protein homodimerization). The analysis of drug aims is among the most recognized techniques for systematically discovering medicines based on ligands. Our bio-active products were found to be exposed to The best docking (binding free energy) scores for all active bio compound were found in the range with 1P7K (Quinoline, 1,2-dihydro-2,2,4-trimethyl -4.6 / amino acid residues PRO:44 (4.11), TYR:36 (4.56) TYR:87 (4.37, 4.87) PHE:98 (4.76, 3.81), Hexadecanoic acid, methyl ester -6.2 kcal/mol/ residues of amino acids interaction LEU:46 (2.18), TYR:36 (4.44, 4.32) PRO:44 (5.02, 5.12), PHE:98 (3.63, 3.89, 4.30, 4.44), TYR:87 (3.99), and 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative = -7.50/ amino acid residues PHE:98 (2.20, 5.29, 4.88, 4.16) TYR:87 (4.16), TYR:36 (4.90) PRO:44 (4.89)) and 5OTE (Quinoline, 1,2-dihydro-2,2,4-trimethyl = -4.2/ residues of amino acid interaction LYS:109 (4.82) VAL:35 (3.89) ASP:32 (2.21) CYS:36 (5.14), Hexadecanoic acid, methyl ester = -6.6/ interaction of amino acid residues SER:221 (2.82), THR:118 (3.70), VAL:90 (5.11) AQE:501 (4.74), PHE:87 (5.15), ALA:86 (4.71), LYS:105 (4.63) and 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative = -8.11/ amino acid interaction THR:239 (2.26) LYS:202 (2.29), ASP:200 (3.66) ASP:218 (4.85) ALA:86 (4.82), AQE:501 (3.91, 2.10) ASP:201 (2.00)). The dock complex exchanges for each ligand were analyzed and inspected with efficient remains.

KEGG and REATOME Pathways Enrichment Analysis

A total of 21 KEGG pathways (*p*-value < 0.05) have been mapped into active compound objectives. The results showed that EREG, EGF, TGFA, GRB2, and HRAS were substantially enhanced throughout the pathway. The number of target mapping pathways was higher than Leukocyte transendothelial migration

(hsa04670 number = 5), Focal adhesion (hsa04510, number = 5), Salmonella infection (hsa05132 number = 5), Regulation of actin cytoskeleton (hsa04810 number=5) and Shigellosis (hsa05131, number = 5). The objectives of active compounds were mapped to twenty-one respire pathways (p -value < 0.05). The findings showed a substantial rise in EREG, EGF, TGFA, and GRB2 in paths. The mapping target paths were greater than the signaling paths are Smooth Muscle Contraction (HSA-445355, number= 3).

Conclusion

To be considerable our studies based on the plants have been found to possess significant biological properties such as antimicrobial, antioxidant and wound healing effects. To scientific evidence of results depicted that *H. binata* leaves extracts were potential sources of essential phytochemicals such as alkaloids, flavonoids, phenols and tannins. The methanolic leaf extract of *H. binata* possessed significant antibacterial activities against certain human bacterial pathogens. Also displayed potential in vitro antioxidant in higher concentration showed scavenging of high free radical and anti-inflammatory activities due to the rich source of bioactive compounds responsible. The ADMET results of *Hardwickia binata* shows pKa, absorption, solubility, bioavailability, BBB penetration. All of the phyto compounds are CYP1A2, CYP2C19, and CYP2C9 inhibitors, as per the CYP interaction. In silico docking of the binding energy, number of hydrogen bond contacts, bond length and the amino acids involved in the interactions. The biomolecules and evaluation of their in vitro and in vivo activity against anti-skin cancer is admirable of further clinical study.

Materials And Methods

Plant collection and extraction

Hardwickia binata

leaves were collected from Shevaroy Hills, Dharmapuri district, Tamil Nadu, India (11.8474° N, 78.2248° E) in November 2020. The collected plant leaves were washed in running tap water and shade dried to 15 days under room temperature. The dried plant leaves were grinded using steel blender and get fine powdered. The powdered plant samples weighed and subjected to soxhlet extraction with hexane, ethyl acetate and methanol. The extract was vacuum dried on rotary shaker (24h at 27°C for each solvent). The extracts were stored at -4°C for further studies. The % of yield was calculated by the formula

$$\text{Percentage of yield} = \frac{\text{Weight of extract residue after solvent removed}}{\text{Weight of dried plant powder}} \times 100$$

2.2 Qualitative analysis of *H. binata*

The *H. binata* leaf extracts were analysed for presence of secondary metabolites. Alkaloids (Mayer's Test), Carbohydrates (Fehling's And Benedict's Test), Phenols (FeCl₃ Test), Saponins (Foam Test), Tannins (FeCl₃ Test), Flavonoids (Lead Acetate Test), Glycosides (Keller- Killani Test), Steroids (Salkowski's Test) Terpenoids (Sulphuric Acid Test) and Proteins (Biuret test) were done using standard procedures [47].

Quantitative analysis

Total Phenolic Content (TPC)

Total Phenolic Content of *H. binata* were quantified by Folin-Ciocalteu Method [48] with slight modification. The 1ml of leaf extract (20, 40, 60, 80, 100 µg/ml) was mixed with 200 µl of 10% Folin-Ciocalteu reagent and allow 2 min at room temperature. After the incubation 10% of 800 µl Na₂CO₃ was subsequently added and kept in dark for 1hr and absorbance was measured at 750 nm. The result was expressed as Gallic acid equivalents in milligrams per gram (mg GAE/g) of dry weight (DW).

Total Flavonoids Content (TFC)

Total Flavonoids Content was evaluated by aluminium chloride colorimetric method [49]. 1ml of extract (50-250 µg/ml) added with 5% of 300 µl sodium nitrate solution and 300 µl of 10% aluminium chloride was added subsequently. Then 4% of 2ml sodium hydroxide added. The mixture was incubate 30 min and absorbance recorded at 435nm. Rutin was as a standard to calculate. Results were expressed as Rutin equivalents in milligrams per gram (mg GAE/g) of dry weight (DW).

Functional group identification of MeOH extract of *H. binata*

The functional groups of plant extracts were identified using FT-IR analysis (Perkin Elmer RXI model). The dried crude extracts were compressed into potassium bromide (KBr) using mortar and pestle, passed through a thin disc under the pressure of 7845 kPa for 2 min, and the absorbance spectra were documented in the range of 4000–400 cm⁻¹.

GC-MS analysis of *H. binata*

The components were separated using helium as the carrier gas at a constant flow rate of 1 ml/min on a fused silica column packed with SH-Rxi- percent Sil MS (30 m 0.25 mm ID 250m df) on a Shimadzu GCMS QP 2020. The injector temperature was set at 280°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 40 °C (2 min); followed by 280 °C at the rate of 10 °C min⁻¹ and 280 °C,

where it was held for 3 min. The mass detector conditions were: transfer line temperature 280 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 550 Da. The component spectrums were compared to a database of known component spectrums recorded in the GC-MS NIST (2017) library.

Biological applications

Antibacterial properties

Staphylococcus aureus, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aureginosa* were collected from microbial culture maintenance laboratory in Department of Microbiology, Periyar University, Salem, Tamil Nadu, India. Antibacterial activity was carried out by disc diffusion method using Gram-positive bacteria - *Enterococcus faecalis*, *staphylococcus aureus* and Gram-negative bacteria - *Escherichia coli*, *Pseudomonas aureginosa*. All bacterial strains are subjected into nutrient broth for sub culturing purpose and broth was procured in test tubes autoclaved at 121°C for 15 min. Individually, all bacterial strains were inoculated in sterile 5ml nutrient broth and incubate at 37°C for 24 hrs. Antibacterial activity of *H. binata* established in disc diffusion method and 100ml of nutrient agar was autoclaved and poured into agar plates and spread evenly. Using a sterilised swab, bacterial strains were swabbed on medium. Three concentrations (50 and 100 µg/mL) were utilised, with *Streptomycin* (10 mcg) using positive control and methanol act as a negative control being placed on the freshly prepared medium. After a 24-hour incubation period at 37°C, an inhibitory zone developed, which was measured in millimetres in diameter [50].

Antioxidant assay of *H. binata* MeOH leaf Extract

DPPH activity of *H. binata*

The extract was diluted to concentrations ranging from 25-125 g/mL in a series of steps. One mL of each dilution was combined with one mL of DPPH solution (0.004% in ethanol) and incubated for 30 minutes at 37°C. Absorbance was recorded at 517 nm using spectrophotometer. Absolute ethanol was used as negative control. Ascorbic acid used as a positive control. By plotting the percent of residual DPPH against the sample concentration, the concentration required for a 50% inhibition of DPPH (IC₅₀) was calculated [51].

ADMET

In Lipinski's rule of five, ADME/T, drug-like properties using compounds with biological and/or pharmacological activity were evaluated, which was used to evaluate if these compounds have the properties that would allow them to be a possibly orally active drug for humans [52].

Molecular Docking

Auto Dock Vina 4.2 has been used to perform docking calculations utilizing the Lamarckian evolutionary algorithms and default procedures to attach a versatile ligand to a stiff protein. Docking computations were done on the binding and catalytic sites of each protein target. Once probable binding sites were identified, compound docking to these sites was done to determine the most likely and energetically desirable binding conformations. To achieve robust docking simulations incorporating a grid box at the identified binding site, AutodockVina4.2 was employed. The completeness of each protein-compound pair was 20. Docking array of 32 A3 with a grid spacing of 0.375A was enclosed by an active site. Affinity scores were obtained and rated based on the free energy binding theory provided by Auto Dock Vina (in kcal/mol) (more negative value means greater binding affinity). To check the relationships use the DS Visualizer 2.5 (<http://3dsbiovia.com/products/>) or 'e PyMOL Molecular Graphics Framework 2.0 programs, the resulting structures, and the binding docking poses were graphically examined [53].

Network Analysis and Enrichment

STRING (<https://string-db.org>, accessed on 7 march 2022) was used to search the list of possible targets. A database for organic evolution, protein kinase, and cellular mechanisms, as well as a protein interaction enrichment study, were created. In addition, the KEGG pathway database was used to distinguish the potentially moderated routes. Plants, phytoconstituents, altered proteins, and structured pathways were all linked in the study. Edge count was used to investigate the complete structure, which was set to "low values to tiny sizes," "low values to vibrant colours," and "low values to tiny sizes."

Statistical Analysis

All of the trials were replicated 3 times, and the data were provided as mean standard deviations (SD). Nonlinear regression curve fit was used to get the IC₅₀, CC₅₀, and EC₅₀ values (GraphPad Prism Software, Version 6 Inc., La Jolla, CA). The criterion of significance was set at 95 percent (p 0.05). Using Microsoft Excel, the correlation coefficients between total phenolics and antioxidant activity methods were validated (2010).

Abbreviations

ADMET - Absorption, Distribution, Metabolism, Elimination and Toxicity, GCMS - Gas Chromatography Mass Spectrometry, CADD - Computer Aided Drug Design, LE - Leaf Extract, ROS - Reactive Oxygen Species, AMR - Antimicrobial Resistance, GAE - Gallic Acid Equivalent, DW - Dry Weight, FTIR- Fourier-Transform Infrared Spectroscopy, *H. binata* - *Hardwickia binata*, DPPH - 2,2-diphenyl-1-picrylhydrazyl, SD - Standard Deviation, mm – millimetre.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Credit Author Statement

KS and PM designed and carried out the experiments, as well as drafting the manuscript. PP and DK were involved in the experiment design and data analysis. SS and M SS corrected and revised the manuscript. The manuscript was critically evaluated by all authors, who contributed to its interpretation. The final version of the manuscript was approved by all writers, and they agreed to be accountable for all aspects of the work.

References

1. Valliammai S, Ashwin MD, Sivachandran B (2020) Evaluation of pharmacognostical, phytochemical and antimicrobial properties of *Clerodendrum heterophyllum* (Poir.) R. br. Mater. Today: Proc. ;33:4377–4384. 10.1016/j.matpr.2020.07.635
2. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177. <https://doi.org/10.3389/fphar.2013.00177>
3. Saddiqe Z, Naeem I, Hellio C, Patel AV, Abbas G (2020) Phytochemical profile, antioxidant and antibacterial activity of four *Hypericum* species from the UK. S Afr J Bot 133:45–53. 10.1016/j.sajb.2020.05.018
4. Batool R, Khan MR, Sajid M, Ali S, Zahra Z (2019) Estimation of phytochemical constituents and in vitro antioxidant potencies of *Brachychiton populneus* (Schott & Endl. BMC Chem Biol 13:1–15. <https://doi.org/10.1186/s13065-019-0549-z>. R. Br
5. Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109:309–318. <https://doi.org/10.1179/2047773215Y.0000000030>
6. Llor C, Bjerrum L (2014) Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf 5:229–241. <https://doi.org/10.1177/2042098614554919>
7. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez L, Salam AM, Shabih S, Farrokhi DR, Quave CL (2021) A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective. Front pharmacol 11:2069. <https://doi.org/10.3389/fphar.2020.586548>
8. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645. <https://dx.doi.org/10.2147%2FIDR.S173867>
9. Kandasamy S, Sahu SK, Kandasamy K (2012) In Silico studies on fungal metabolite against skin cancer protein (4, 5-Diarylisoaxazole HSP90 Chaperone). int. sch. res. Notices. doi: 10.5402/2012/626214
10. Sarkar B, AsadUllah MD, Sajidul Islam S (2020) Silico analysis of some phytochemicals as potential anti-cancer agents targeting cyclin dependent kinase-2, human topoisomerase and vascular endothelial growth factor receptor-2. Bio Rxiv 2020–2001. Doi: 10.1080/10799893.2020.1805628
11. Anza M, Endale M, Cardona L, Cortes D, Eswaramoorthy R, Zueco J, Rico H, Treliis M, Abarca B (2021) Antimicrobial Activity, in silico Molecular Docking, ADMET and DFT Analysis of Secondary Metabolites from Roots of Three Ethiopian Medicinal Plants. Adv Appl Bioinform Chem 14:117. <https://dx.doi.org/10.2147%2FAABC.S323657>
12. Shingade SP, Kakde RB (2021) A Review on “Anjan” *Hardwickia binata* Roxb: Its Phytochemical Studies, Traditional uses and Pharmacological activities. Pharmacogn Rev 15:65. 10.5530/phrev.2021.15.7
13. Deshmukh SV, Ghanawat NA (2019) Phytochemical studies, FTIR and GC-MS analysis of *Hardwickia binata* Roxb. (Fabaceae/Caesalpinaceae). Int J Pharm Sci 1:233–240. DOI: 10.13040/IJPSR.0975-8232
14. Rageeb M, Usman M, Patil R (2022) Antimicrobial and antifungal activity of bark of *Hardwickia binata* roxb (fabaceae / caesalpinaceae). Int J Pharm Sci & Res 13:1189–1193. doi: 10.13040/IJPSR.0975-8232
15. Zhang L, Zengin G, Rocchetti G, Senkardes I, Sharmeen JB, Mahomoodally MF, Behl T, Roupahel Y, Lucini L (2021) Phytochemical Constituents and Biological Activities of the Unexplored Plant *Rhinanthus angustifolius* subsp. grandiflorus. Appl Sci 11:9162. <https://doi.org/10.3390/app11199162>
16. Loganathan S, Selvam K, Sivasakthi V, Prakash P, Yamuna M, Lalitha K, Shivakumar MS, Senthil Nathan S (2021) Phytochemical and Pharmacological Evaluation of Methanolic Extract of *Knoxia sumatrensis* Leaves. J Herbs Spices Med Plants 27(2):200–217
17. Gacem MA, Telli A, Gacem H, Ould-El-Hadj-Khelil A (2019) Phytochemical screening, antifungal and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies). SN Appl Sci 1:1–13. <https://doi.org/10.1007/s42452-019-1797-1>
18. Abbas A, Naqvi SAR, Rasool MH, Noureen A, Mubarik MS, Tareen RB (2021) Phytochemical Analysis, Antioxidant and Antimicrobial Screening of *Seriphidium Oliverianum* Plant Extracts. DOSE-RESPONSE 19:15593258211004739. <https://doi.org/10.1177%2F15593258211004739>
19. Ayoub L, Hassan F, Hamid S, Abdelhamid Z, Souad A (2019) Phytochemical screening, antioxidant activity and inhibitory potential of *Ficus carica* and *Olea europaea* leaves. Bioinformation 15:226. <https://dx.doi.org/10.6026%2F97320630015226>
20. Mehwish S, Islam A, Ullah I, Wakeel A, Qasim M, Khan MA, Ullah N (2019) In vitro antileishmanial and antioxidant potential, cytotoxicity evaluation and phytochemical analysis of extracts from selected medicinally important plants. Biocatal Agric Biotechnol 19:101117. <https://doi.org/10.1016/j.bcab.2019.101117>

21. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV (2016) An overview on the role of dietary phenolics for the treatment of cancers. *Nutr. J.* ;15:1–16. <https://doi.org/10.1186/s12937-016-0217-2>
22. Pereira DM, Valentão P, Pereira JA, Andrade PB (2009) Phenolics: From chemistry to biology. *mol* 14:2202–2211. doi: 10.3390/molecules14062202
23. Saeed N, Khan MR, Shabbir M (2012) Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern* 12:1–12. <https://doi.org/10.1186/1472-6882-12-221>
24. Sunil KS, Suma A, Ashika BD, Roy CL, Nareesh S, Sathyamurthy B (2018) GCMS And FTIR Analysis on the Methanolic Extract of *Coriandrum Sativum* Leaves. *Eur J Pharm Sci EUR J PHARM SCI* 5:454–460
25. Singh N, Mansoori A, Jiwani G, Solanke AU, Thakur TK, Kumar R, Chaurasiya M, Kumar A (2021) Antioxidant and Antimicrobial Study of *Schefflera vinosa* Leaves Crude Extracts Against Rice Pathogens. *Arab J Chem* 103243. 10.1016/j.arabjc.2021.103243
26. Saha S, Acharya M (2014) Hydrazinecarboxamide or hydrazinecarbothioamide Bearing small molecules as Dual inhibitor of Ras protein and Carbonic anhydrase enzyme as potential anticancer agent—A MLR approach based on docking energy. *Res J Biotechnol* 5:15–23
27. Choi D, Kang W, Park T (2020) Anti-allergic and anti-inflammatory effects of Undecane on mast cells and keratinocytes. *Mol* 25:1554. <https://doi.org/10.3390/molecules25071554>
28. Das S, Vasudeva N, Sharma (2014) S Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy. *Bioorganic Med. Chem. Lett.* ;4:1–4. <http://www.orgmedchemlett.com/content/4/1/13>
29. Tyagi T, Agarwal M (2017) Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *pistia stratiotes* L. *Int j basic appl med* 7:14–21
30. Begum IF, Mohankumar R, Jeevan M, Ramani K (2016) GC–MS analysis of bio-active molecules derived from *Paracoccus pantotrophus* FMR19 and the antimicrobial activity against bacterial pathogens and MDROs. *Indian J Microbiol* 56:426–432. doi: 10.1007/s12088-016-0609-1
31. Ruksana MS, Doss A, Kumari PR (2017) Phytochemical screening and GC-MS analysis of leaf extract of *Pergularia daemia* (Forssk) Chiov. *Asian J Plant Sci Res* 7:9–15
32. Arora S, Meena S (2017) GC-MS Profiling of *Ceropegia bulbosa* Roxb. var. bulbosa, an endangered plant from Thar Desert. *Rajasthan J Pharm Innov* 6:568–573
33. Silva SADNM, Barros AB, Souza JMT, Moura AF, de Araujo AR, Mendes MGA, Daboit TC, da Silva D, Araujo AJ, Marinho Filho JDB (2020) Phytochemical and biological prospection of Mimosa genus plants extracts from Brazilian northeast. *Phytochem Lett* 39:173–181. 10.1016/j.phytol.2020.08.010
34. Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S, Jodie D (2018) Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *J King Saud Univ Sci* 30:500–505. <https://doi.org/10.1016/j.jksus.2017.03.009>
35. Vignesh A, Selvakumar S, Vasanth K (2021) Comparative LC-MS analysis of bioactive compounds, antioxidants and antibacterial activity from leaf and callus extracts of *Saraca asoca*. *Phytomedicine Plus* 100167. <https://doi.org/10.1016/j.phyplu.2021.100167>
36. Bajpai VK, Rahman A, Shukla S, Mehta A, Shukla S, Arafat SY, Rahman MM, Ferdousi Z (2009) Antibacterial activity of leaf extracts of *Pongamia pinnata* from India. *Pharm Biol* 47:1162–1167. <https://doi.org/10.3109/13880200903019218>
37. Khan UA, Rahman H, Niaz Z, Qasim M, Khan J, Tayyaba Rehman B (2013) Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *Eur J Immunol* 3:272–274. <https://doi.org/10.1556/eujmi.3.2013.4.6>
38. Flieger J, Flieger M (2020) The [DPPH●/DPPH-H]-HPLC-DAD Method on Tracking the Antioxidant Activity of Pure Antioxidants and Goutweed (*Aegopodium podagraria* L.) Hydroalcoholic Extracts. *Mol* 25:6005. <https://doi.org/10.3390/molecules25246005>
39. Ahmed F, Iqbal M (2018) Antioxidant activity of *Ricinus Communis*. *Org Biomol Chem* 5:107–112. DOI: 10.19080/OMCIJ.2018.05.555667
40. Nair RVR, Varma K, Paul B, Amalraj A, Kuttappan S (2021) Evaluation of the anti-hyperglycemic and antioxidant activities of a novel phytochemical formulation. *Phytomedicine Plus* 1:100093. <https://doi.org/10.1016/j.phyplu.2021.100093>
41. Bruck de Souza L, Leitão Gindri A, de Andrade Fortes T, Felli Kubiça T, Enderle J, Roehrs R, Moura e Silva S, Manfredini V, Gasparotto Denardin EL (2020) Phytochemical Analysis, Antioxidant Activity, Antimicrobial Activity, and Cytotoxicity of *Chaptalia nutans* Leaves. *Adv Pharmacol Sci*. doi: 10.1155/2020/3260745
42. Rahim NA, Zakaria N, Dzulkarnain SMH, Azahar NMZM, bdulla MA (2017) Antioxidant activity of *alstonia Angustifolia* ethanolic leaf extract. In *AIP Conference Proceedings* (Vol. 1891, No. 1, p. 020012). AIP Publishing LLC. <https://doi.org/10.1063/1.5005345>
43. Alam S, Khan F (2018) Virtual screening, Docking, ADMET and System Pharmacology studies on Garcinia caged Xanthone derivatives for anticancer activity. *Sci Rep* 8:1–16. <https://doi.org/10.1038/s41598-018-23768-7>
44. Daoui O, Elkhatabi S, Chtita S, Elkhatabi R, Zgou H, Benjelloun AT (2021) QSAR, molecular docking and ADMET properties in silico studies of novel 4, 5, 6, 7-tetrahydrobenzo [D]-thiazol-2-Yl derivatives derived from dimedone as potent anti-tumor agents through inhibition of C-Met receptor tyrosine kinase. *Heliyon*. ;7:e07463. <https://doi.org/10.1016/j.heliyon.2021.e07463>
45. Alam MS, Ahmed JU, Lee DU (2016) Biological features, drug-likeness, pharmacokinetic properties, and docking of 2-arylidenehydrazinyl-4-arylthiazole analogues. *Appl Biol Chem* 59:181–192. <https://doi.org/10.1007/s13765-016-0148-9>
46. Chinthala Y, Thakur S, Tirunagari S, Chinde S, Domatti AK, Arigari NK, Srinivas KVNS, Alam S, Jonnala KK, Khan F, Tiwari A (2015) Synthesis, docking and ADMET studies of novel chalcone triazoles for anti-cancer and anti-diabetic activity. *Eur J Med Chem* 93:564–573. <https://doi.org/10.1016/j.ejmech.2015.02.027>
47. Mahendra C, Manasa G, Kiran BL, Murali M, Girish HV, Sudarshana MS (2017) Phytochemical, Histochemical, and Antibacterial Screening of *Chenopodium anthelminticum* L. *J. Herbs Spices Med. Plants* 23:183–192. <https://doi.org/10.1080/10496475.2017.1295123>

48. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants* 8:96. doi: 10.3390/plants8040096
49. Kamtekar S, Keer V, Patil V (2014) Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *J appl pharm* 4:61. DOI: 10.7324/JAPS.2014.40911
50. Mohamed EAA, Muddathir AM, Osman MA (2020) Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two *Pulicaria* spp. *Grow Sudan Sci Rep* 10:1–8. <https://doi.org/10.1038/s41598-020-74262-y>
51. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC (2019) Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *J Food Qual.* <https://doi.org/10.1155/2019/8178294>
52. Prakash P, Archana G, Gayathiri E, Mani V, Manivasagaperumal R, Vinoth B, Peele KA, Selvam K, Thirupathi A, Alodaini HA, Al-Dosary MA (2022) Pharmacokinetics studies, molecular docking and discovery of anti-proliferative agents and its targeting EGFR inhibitors. *J. King Saud Univ Sci* 34:101679. <https://doi.org/10.1016/j.jksus.2021.101679>
53. Nirmalraj S, Gayathiri E, Sivamurugan M, Manivasagaperumal R, Jayanthi J, Prakash P, Selvam K (2021) Molecular docking based screening dynamics for plant based identified potential compounds of PDE12 inhibitors. *CRGSC* 100122. <https://doi.org/10.1016/j.crgsc.2021.100122>

Tables

Table 1 FTIR- Fourier transform infrared spectroscopy methanolic leaves extract of *Hardwickia binata*

S. No	Wave number (cm ⁻¹)	Intensity	Bond	Group Compound	Functional Group
1	667.70	Strong	Bending	C=C	Alkene
2	1053.53	Strong	Stretching	C=O	Primary Alcohol
3	1455.35	Medium	Bending	C-H	Alkene
4	1623.27	Medium	Stretching	C=C	Conjugated Alkene
5	2362.94	Strong	Stretching	O=C=O	Carbon Dioxide
6	2916.70	Medium	Stretching	C-H	Alkene
7	3370.50	Medium	Stretching	N-H	Aliphatic Primary amine

Table 2 Antibacterial activity of the methanol extract of *H. binata* leaves extract (zone of inhibition mm)

S. No	Pathogens	Zone of inhibition (mm) methanol leaf extract (µg/ml)			
		Positive control (Streptomycin)	Negative control (methanol)	methanol leaf extract (50 µl)	methanol leaf extract (100 µl)
1	<i>Staphylococcus aureus</i>	14 ± 1.22	-	10 ± 2.12	12 ± 2.63
2	<i>Enterococcus faecalis</i>	15 ± 1.23	07 ± 2.11	13 ± 3.11	14 ± 2.54
3	<i>Escherichia coli</i>	11 ± 2.11	16 ± 2.23	05 ± 4.23	07 ± 3.31
4	<i>Pseudomonas aureginosa</i>	13 ± 1.14	08 ± 3.12	08 ± 5.21	10 ± 3.35

Table 3 In Silico predicted physiochemical properties of 27 plant derivate.

S. No	Compound Name	MF	Heavy atoms	Aromatic heavy atoms	Fraction Csp3	Rotatable bonds	H-bond accepters	H-bond donors	TPSA
1.	Hydrazinecarbothioamide	CH ₅ N ₃ S	5	0	0	1	1	3	96.16
1.	Undecane	C ₁₁ H ₂₄	11	0	1	8	0	0	0
1.	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	C ₁₂ H ₁₅ N	13	6	0.33	0	0	1	12.03
1.	Phenol, 2,4-Bis (1,1-Dimethylethyl)-	C ₂₂ H ₃₀ O	23	12	0.45	4	1	1	20.23
1.	Tetratriacontane	C ₃₄ H ₇₀	34	0	1	31	0	0	0
1.	Heneicosane	C ₂₁ H ₄₄	21	0	1	18	0	0	0
1.	Heneicosane	C ₂₁ H ₄₄	21	0	1	18	0	0	0
1.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	19	0	0.94	15	2	0	26.3
1.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	18	0	0.94	14	2	1	37.3
1.	Eicosane	C ₂₀ H ₄₂	20	0	1	17	0	0	0
1.	Manganese, [[1,2-Ethanediy]bis[Carbam]	C ₄ H ₆ MnN ₂ S ₄	11	0	0.5	5	0	2	88.24
1.	Hexadecanoic acid, 16-methyl-,methyl ester	C ₂₁ H ₄₀ O ₄	25	0	0.9	18	4	0	52.6
1.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	20	0	0.94	16	2	1	37.3
1.	Eicosane	C ₂₀ H ₄₂	20	0	1	17	0	0	0
1.	1-Hexadecanol, Acetate	C ₁₈ H ₃₆ O ₂	20	0	0.94	16	2	0	26.3
1.	Tetracosane	C ₂₄ H ₅₀	24	0	1	21	0	0	0
1.	Hexatriacontane	C ₃₆ H ₇₄	36	0	1	33	0	0	0
1.	1-Heptacosanol	C ₂₇ H ₅₆ O	28	0	1	25	1	1	20.23
1.	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	40	0	1	0	10	0	92.3
1.	Bis (2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	28	6	0.67	16	4	0	52.6
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	48	0	1	0	12	0	110.76
1.	Dotriacontane	C ₃₂ H ₆₆	32	0	1	29	0	0	0
1.	4,4'-((p-Phenylene)diisopropylidene) diphenol	C ₂₄ H ₂₆ O ₂	26	18	0.25	4	2	2	40.46
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	48	0	1	0	12	0	110.76
1.	1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	28	6	0.67	16	4	0	52.6
1.	5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative	C ₁₆ H ₂₆ N ₄ Si ₂	22	12	0.38	5	2	2	49.84
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	48	0	1	0	12	0	110.76

Table 4 ADME predicted physiochemical properties for 27 plant derivate

S. No	Compound Name	MF	Molecular Weight	Absorption	CYP1A2	CYP2C19	CYP2C9	CYP2D6	C
1.	Hydrazinecarbothioamide	CH ₅ N ₃ S	91.14	High	No	No	No	No	N
1.	Undecane	C ₁₁ H ₂₄	156.31	Low	Yes	No	No	No	N
1.	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	C ₁₂ H ₁₅ N	173.25	High	No	No	No	Yes	N
1.	Phenol, 2,4-Bis (1,1-Dimethylethyl)-	C ₂₂ H ₃₀ O	310.5	High	No	Yes	No	Yes	N
1.	Tetratriacontane	C ₃₄ H ₇₀	478.9	Low	No	No	No	No	N
1.	Heneicosane	C ₂₁ H ₄₄	296.6	Low	Yes	No	No	No	N
1.	Heneicosane	C ₂₁ H ₄₄	296.6	Low	Yes	No	No	No	N
1.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5	High	Yes	No	No	No	N
1.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	High	Yes	No	Yes	No	N
1.	Eicosane	C ₂₀ H ₄₂	282.5	Low	Yes	No	No	No	N
1.	Manganese, [[1,2-Ethanediy]bis[Carbam]	C ₄ H ₆ MnN ₂ S ₄	265.3	High	No	No	No	No	N
1.	Hexadecanoic acid, 16-methyl-,methyl ester	C ₂₁ H ₄₀ O ₄	356.5	High	Yes	No	Yes	No	Y
1.	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5	High	Yes	No	No	No	N
1.	Eicosane	C ₂₀ H ₄₂	282.5	Low	Yes	No	No	No	N
1.	1-Hexadecanol, Acetate	C ₁₈ H ₃₆ O ₂	284.5	High	Yes	No	No	No	N
1.	Tetracosane	C ₂₄ H ₅₀	338.7	Low	Yes	No	No	No	N
1.	Hexatriacontane	C ₃₆ H ₇₄	507	Low	No	No	No	No	N
1.	1-Heptacosanol	C ₂₇ H ₅₆ O	396.7	Low	No	No	No	No	N
1.	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	741.5	Low	No	No	No	No	N
1.	Bis (2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.6	High	No	No	Yes	No	Y
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	889.8	Low	No	No	No	No	N
1.	Dotriacontane	C ₃₂ H ₆₆	450.9	Low	No	No	No	No	N
1.	4,4'-((p-Phenylene)diisopropylidene) diphenol	C ₂₄ H ₂₆ O ₂	346.5	High	No	Yes	No	Yes	N
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	889.8	Low	No	No	No	No	N
1.	1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.6	High	No	No	Yes	No	Y
1.	5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative	C ₁₆ H ₂₆ N ₄ Si ₂	330.57	High	Yes	Yes	No	Yes	Y
1.	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	889.8	Low	No	No	No	No	N

Table 5 Binding energy, number of hydrogen bond contacts, bond length and the amino acids involved in the interactions

S. No	Compound Name	Binding Energy		Amino Acid Interaction	
		50TE	1P7K	50TE	1P7K
1	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	-4.2	-4.6	LYS:109 (4.82) VAL:35 (3.89) ASP:32 (2.21)CYS:36 (5.14)	PRO:44 (4.11),TYR:36 (4.56)TYR:87 (4.37, 4.87) PHE:98 (4.76, 3.81)
2	Hexadecanoic acid, methyl ester	-6.6	-6.2	SER:221 (2.82), THR:118(3.70),VAL:90 (5.11) AQE:501 (4.74),PHE:87 (5.15),ALA:86 (4.71), LYS:105 (4.63)	LEU:46 (2.18),TYR:36 (4.44, 4.32) PRO:44 (5.02, 5.12),PHE:98 (3.63, 3.89, 4.30, 4.44),TYR:87 (3.99)
3	5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative	-8.11	-7.50	THR:239 (2.26) LYS:202 (2.29),ASP:200(3.66)ASP:218(4.85)ALA:86 (4.82),AQE:501 (3.91, 2.10)ASP:201 (2.00)	PHE:98 (2.20, 5.29, 4.88, 4.16)TYR:87(4.16),TYR:36 (4.90)PRO:44 (4.89)

Figures

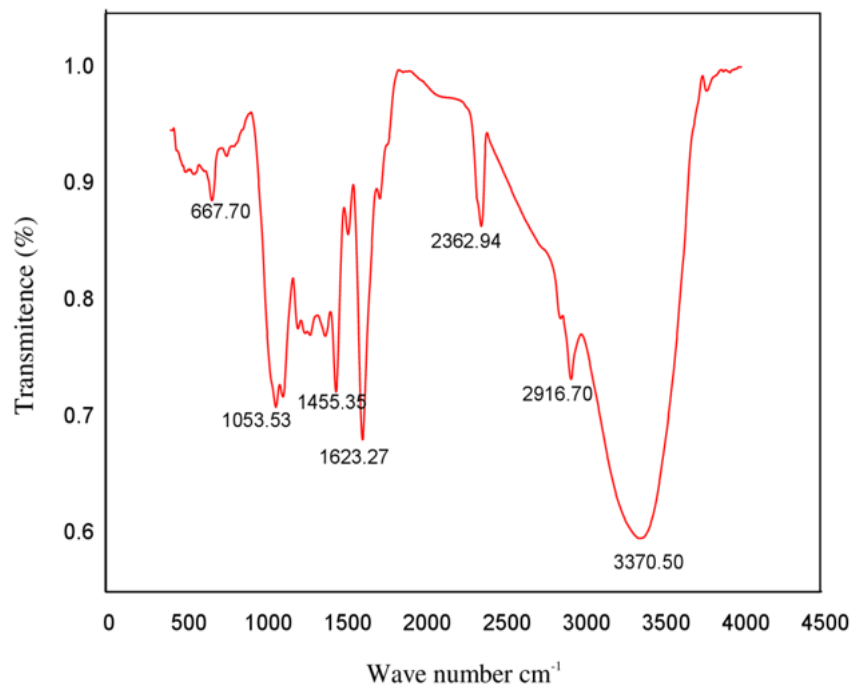


Figure 1

FTIR spectrum analysis of methanolic leaves extract of *Hardwickia binata* and their probable functional groups with wavenumbers. N–H, C–H, C=O functional groups were observed which designate the presence of various metabolites in plant extracts.

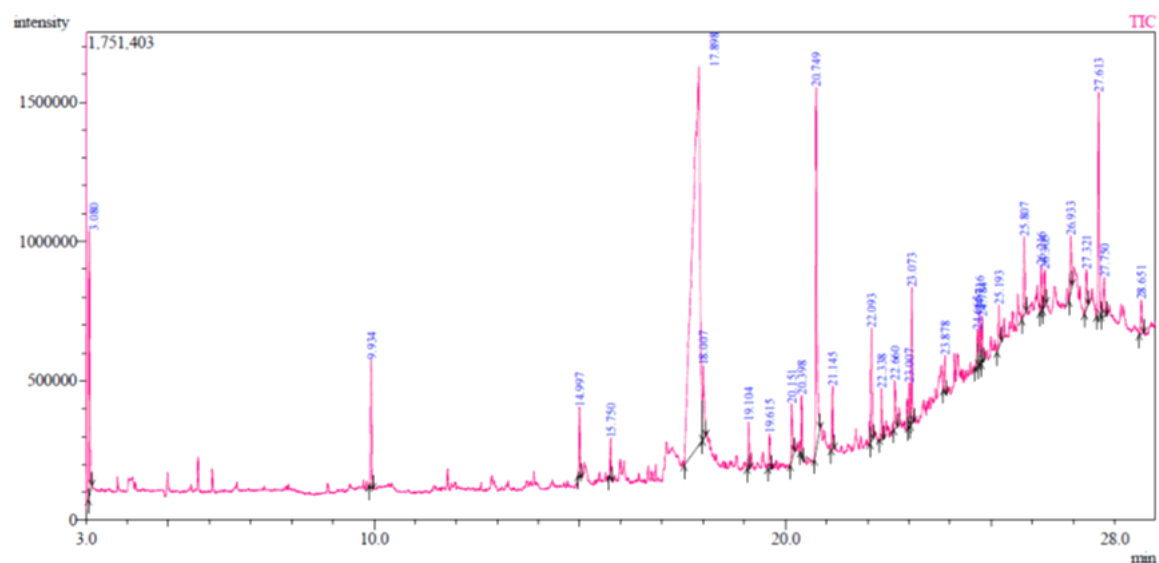


Figure 2

Chemical composition of methanolic extract of *Hardwickia binata* leaf extract.

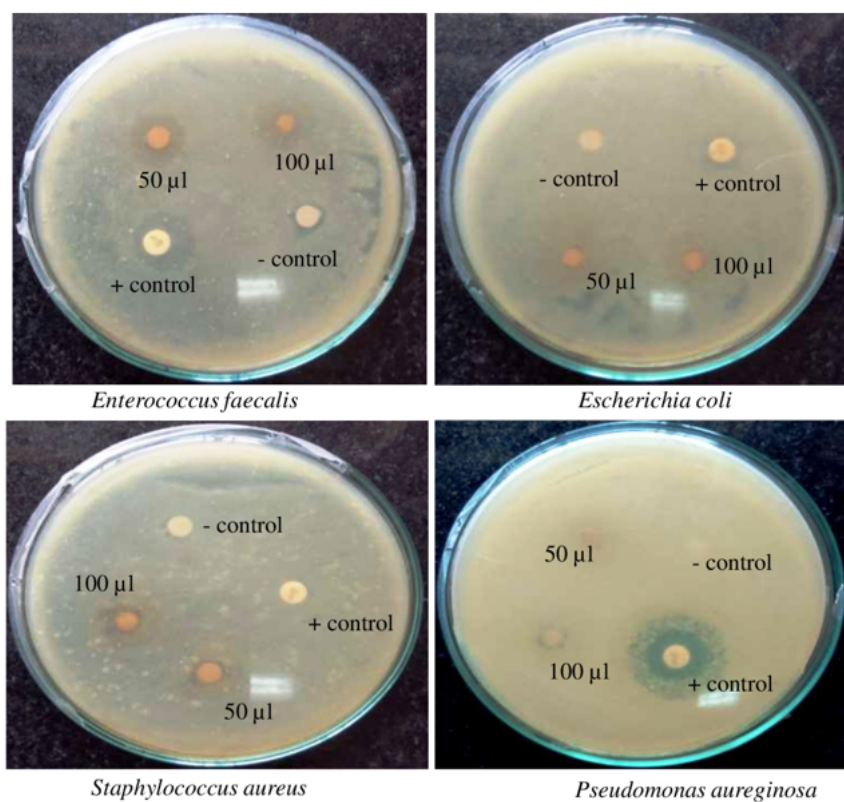


Figure 3

Antibacterial activity of *H. binata* crude extract against human pathogens (50 µl, 100 µl, Positive control - *Streptomycin*, Negative control - Methanol).

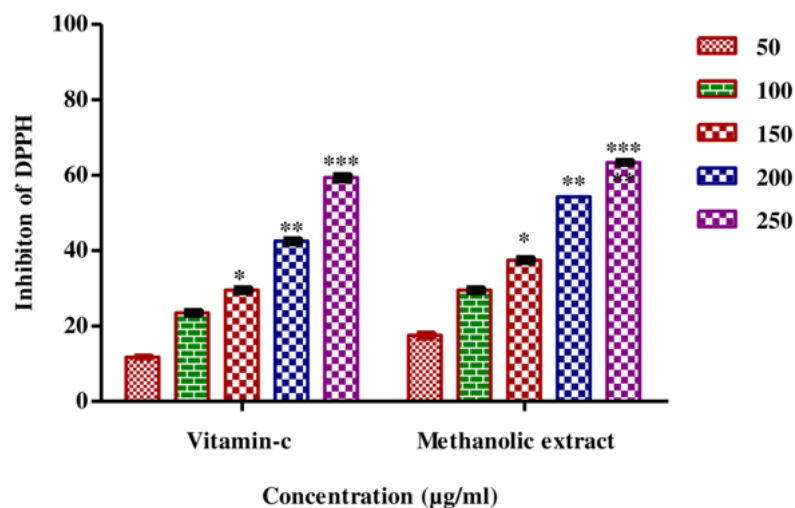


Figure 4

antioxidant activity (DPPH Assay) of *H. binata* methanolic leaf extract in different concentration. The values expressed as mean $\pm$ SD values, analyzed by Two-way analysis of Variance (ANOVA). Followed by Dunnett's multiple comparison tests ($P < 0.06$; $P < 0.001$; $P < 0.0001$). Asterisk (*) (**) (***) indicates significance differences among treatments compared in control groups.

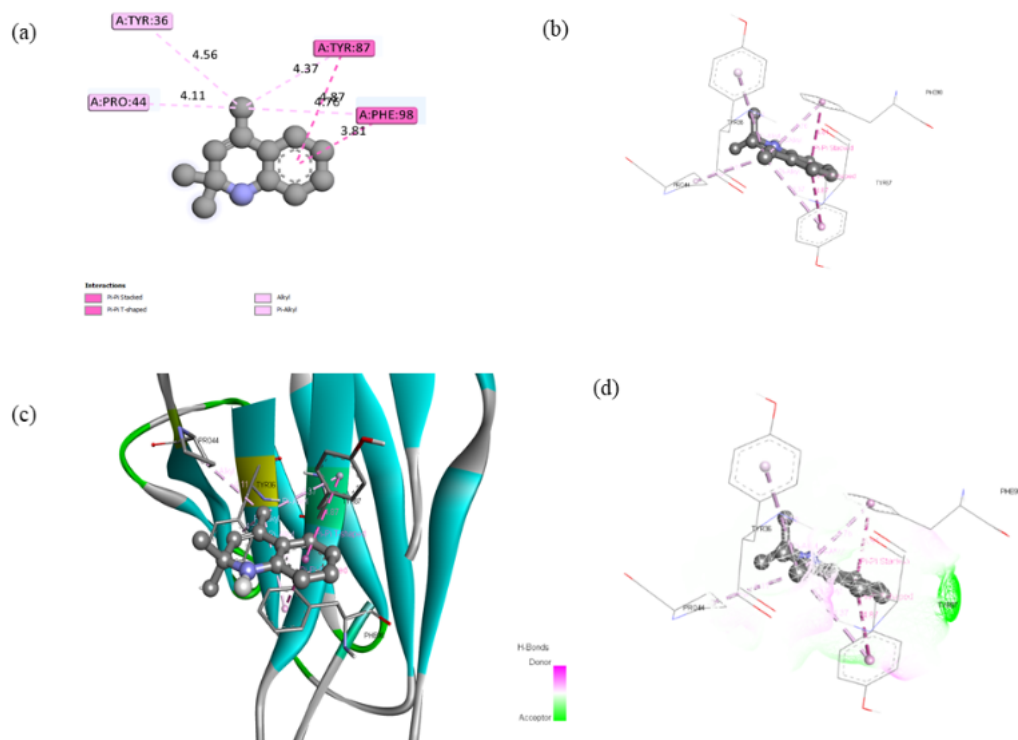


Figure 5

(a-d) The interaction of the 2D ligand (Quinoline, 1,2-dihydro-2,2,4-trimethyl-) with protein 1P7K, a) 2D ligand interaction, b) 3D ligand complex interaction c) 3D complex interaction d) 3D Hydrogen surface of complex with the possible H-bond donor and acceptor groups.

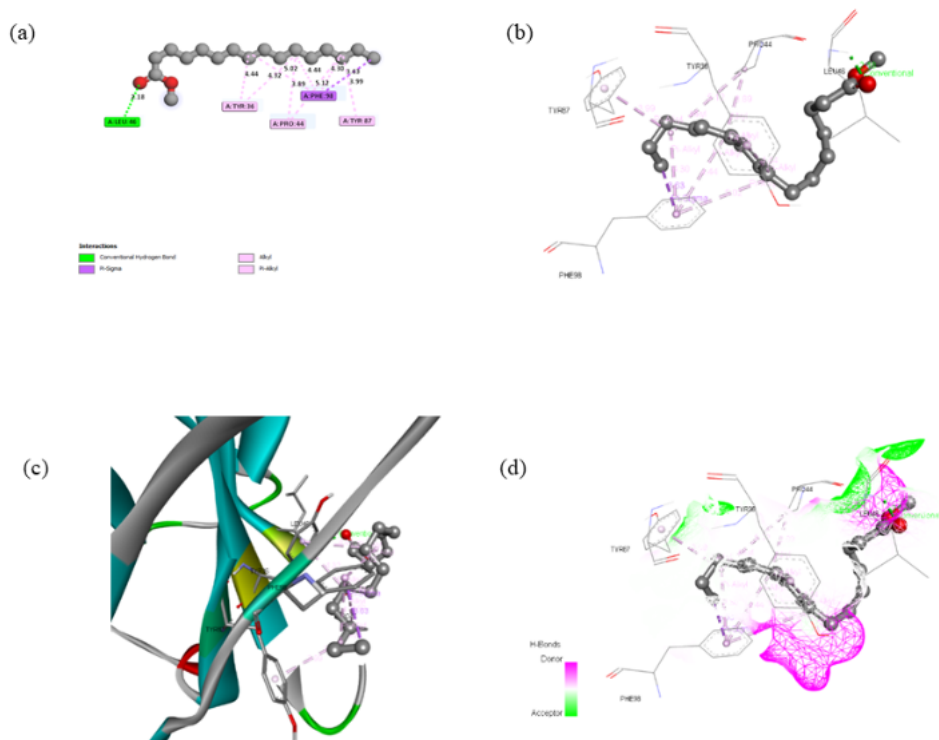


Figure 6

(a-d) 2D and 3D docking interactions between (Hexadecanoic acid, methyl ester) with protein 50TE, a) 2D ligand interaction, b) 3D complex interaction, c) 3D complex interaction d) 3D Hydrogen surface of complex with the possible H-bond donor and acceptor groups.

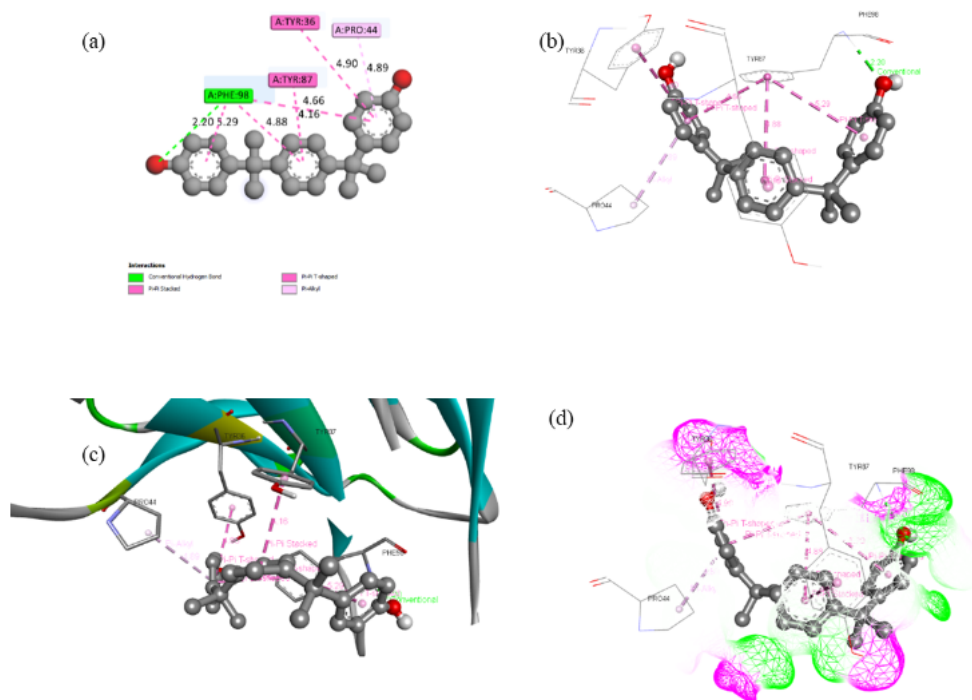


Figure 7

(a-d) Docking structure of 5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative bound with 1P7K protein fragment in presence of (a) The interaction of the 2D ligand, (b) The 3D structure of the ligand with protein, c) 3D complex interaction d) The interaction of the ligand with protein 1P7K with the possible H-bond donor and acceptor groups.

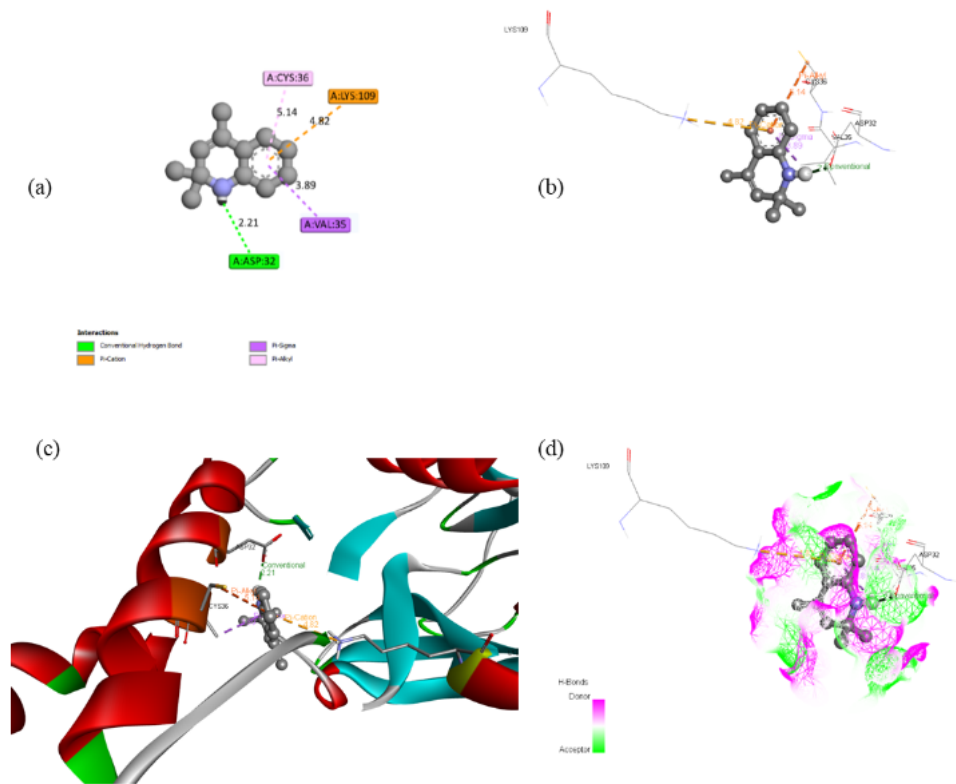


Figure 8

(a-d) 2D and 3D Molecular docking interactions between (Quinoline, 1,2-dihydro-2,2,4-trimethyl-) with protein 50TE, a) 2D ligand interaction, b) 3D complex interaction, c) 3D complex interaction d) 3D Hydrogen surface of complex with the possible H-bond donor and acceptor groups.

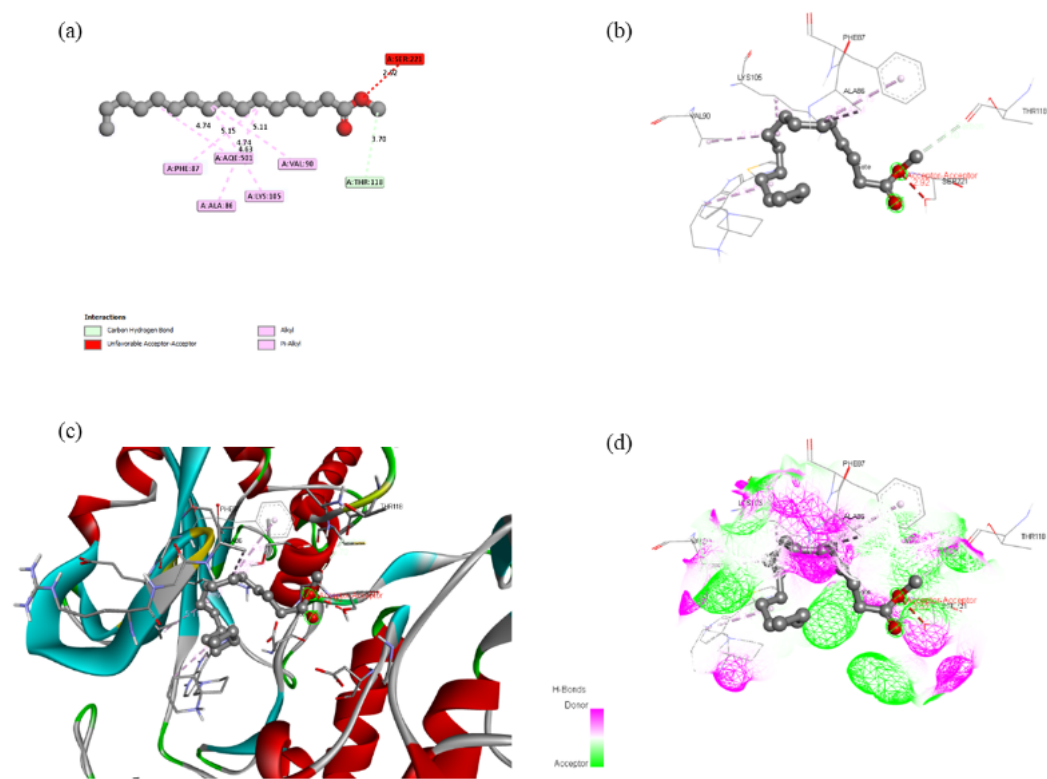


Figure 9

(a-d) 2D and 3D docking interactions between (Hexadecanoic acid, methyl ester) with protein 50TE, a) 2D ligand interaction, b) 3D complex interaction, c) 3D complex interaction d) 3D Hydrogen surface of complex with the possible H-bond donor and acceptor groups.

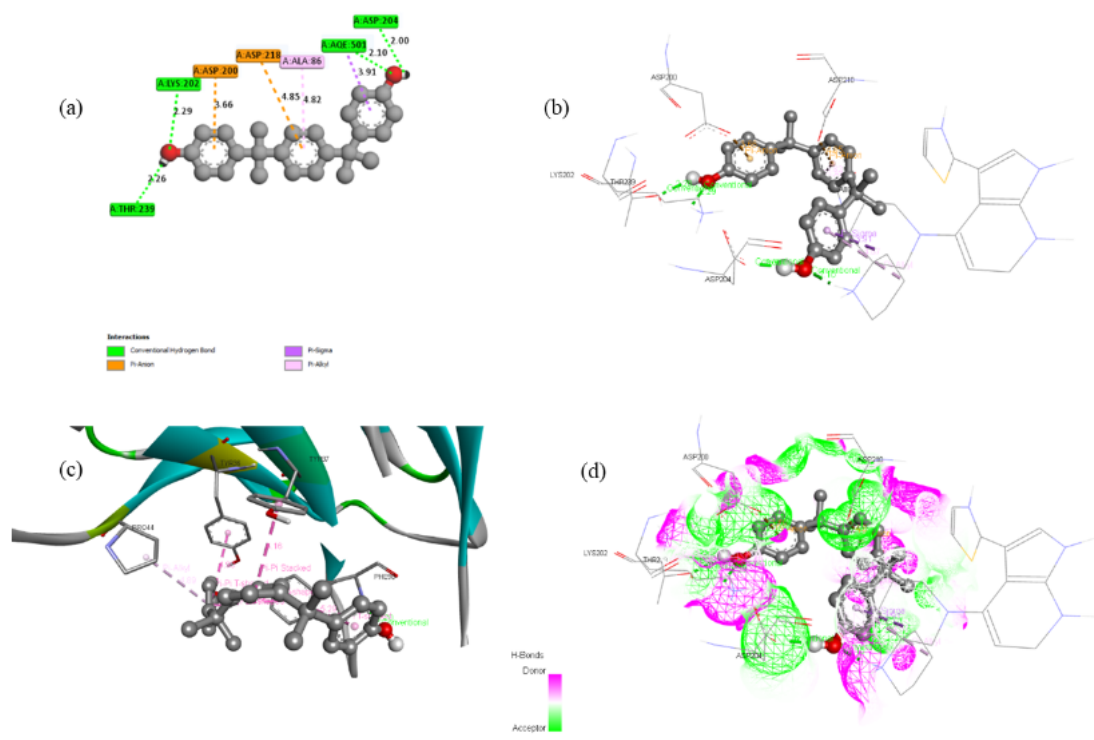


Figure 10

(a-d) 2D and 3D Ligand-receptor interactions of compounds (5-Phenyl-2,4-pyrimidinediamine, 2TMS derivative) with 50TE a) 2D ligand interaction, b) 3D complex interaction, c) 3D complex interaction d) 3D Hydrogen surface of complex with the possible H-bond donor and acceptor groups.

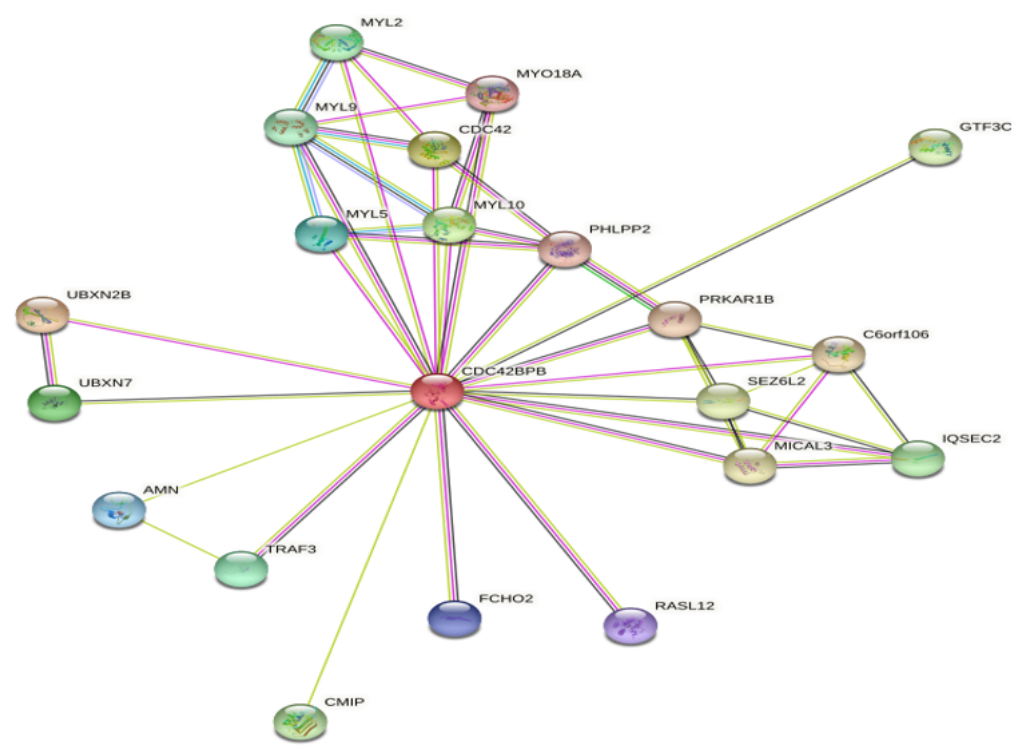


Figure 11

Protein–protein interaction (PPI) networks of bioactive components of *Hardwickia binata* for the treatment of skin cancer. Each node represents the relevant gene, the edge means line thickness indicates the strength of data support. Protein-protein interaction network of targets related to cancer. The colored nodes represent candidate proteins and colored lines represent protein interactions. Light green - represents text mining; Black - represents co-expression; Light blue- represents known interactions from curated databases; State blue - represents protein homology; Magenta- represents experimentally determined known interactions; Green - represents predicted interactions between neighbourhood genes; Red - represents predicted interactions of gene fusion; and Blue – represents predicted interactions between co-occurred genes.

Supplementary Files

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- [supplementary.docx](#)