

CHEMICAL COMPOSITION, ANTIMICROBIAL AND DOCKING STUDIES OF CHLOROFORM EXTRACTS OF *Piliostigma thonningii* LEAVES AND SEEDS

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ABSTRACT

Piliostigma thonningii is one of the African's medicinal plants that is used in ethnomedicine for wound healing and treatment of inflammations, bacterial infections, diarrhoea, worms' infestation, stomach ache and headache. Chemical studies of the chloroform extracts of the leaves and seeds of the plant using GC-MS technique revealed the presence of 18 and 9 compounds respectively. The antibacterial activity screening of the extracts were studied using hospital isolates of five pathogenic bacteria organisms. Its activity at a varying concentrations of 150, 75, 50, 25, 12.5 and 6.25 mg/ml was investigated against *S. aureus*, *B. cereus*, *E. coli*, *S. typhi* and *P. aeruginosa* using agar well diffusion method. The result showed that the extract of the leaf exhibited activity against three micro-organisms in a concentration-dependent pattern, with the highest growth inhibition seen in *S. aureus* and the lowest growth in *S. typhi* with MIC of 25 and 75 mg/ml respectively while the seed showed inhibition activity against two microorganisms in a concentration-dependent manner, with the highest growth inhibition seen in *S. aureus* and the lowest growth in *E. coli* with MIC of 50 and 75 mg/ml respectively. The result of this research work revealed that *P. thonningii* has a lot of phytochemicals which might be responsible for its therapeutic potentials in ethnomedicine. The compounds exhibited negative binding affinities in the molecular docking studies. This suggests the potential of the compounds to inhibit the growth of the bacteria species. The results emphasize the therapeutic potential of *P. thonningii* leaves and seeds extract as a natural source of bioactive compounds with antibacterial activities. This highlights the need for more research to explore its potential use in pharmaceutical applications.

Keywords: *Piliostigma thonningii*; Phytochemicals; GC-MS; Antimicrobial; Molecular docking; Chloroform extract

INTRODUCTION

Medicinal plants are considered as rich sources of constituents that can be used in drug development and synthesis. Many cultures still rely on indigenous medicinal plants that have been used by traditional medicine practitioners [1, 2]. Herbal medicine is now globally accepted as a valid alternative system of therapy in the form of pharmaceuticals, food supplements,

nutraceuticals, complementary and alternative medicines [3]. These medicinal herbs contain many active principles that are used to treat diseases and relieve symptoms. Nigeria is one of the countries in the world that is blessed with a lot of medicinal plants in which *Piliostigma thonningii* is one of them.

Piliostigma thonningii is a leguminous plant that belongs to the *Fabaceae* family which includes trees, shrubs, and perennial or annual herbaceous plants that are easily recognized by their fruits (legume) and their compound, stipulated leaves [4, 5]. The plant, commonly known as camel's foot tree or monkey bread (English), okpoatu (Igbo), abafe (Yoruba) and kalgo (Hausa), grows up to 4 – 15 m in height with a rounded crown and a short but often crooked bole [6]. *P. thonningii* is native to tropical African countries such as the Sudano-Guinean region, Senegal, Namibia, Botswana, Nigeria, Mozambique and South Africa where it is found commonly in the open woodland and wooded grasslands at medium to low altitudes [6].

The use of *P. thonningii* in traditional medicine has been intensively reported. The leaves and the bark of the plant are used in the treatment of malaria, wounds, ulcers, gastric/heart pain, gingivitis, fever, haemorrhoids and backache [4,7]. The plant has been touted to be used in the treatment of diseases such as cough, gonorrhea, snake bite, ethno-veterinary diseases, especially across Africa. Pharmacological studies have shown the plant to possess antimalarial, antiviral, antimicrobial and anti-proliferative activities [8]. A decoction of the leaf is drunk and bathed against fever, toothache, and epilepsy. It is also used as a vaginal wash and as an enema to a mother giving birth [9]. The fresh leaves and flowers of this tree can be chewed to reduce thirst. Bark infusions are used to treat diarrhoea. The

leaf preparations are antiseptic and are used to promote wound healing, treat skin diseases and snake bites [9]. The medicinal uses of *P. thonningii* also include treating loose stool in teething children, worm infestation, arresting bleeding, inflammations, bacterial infections, stomach ache, headache and infertility [10].

Computer-aided methods for drug development have advanced as enhanced technologies that can be employed to analyze pharmaceuticals created from phytochemicals found in various medicinal plants. Computational prediction models play a crucial role in directing the selection of methodologies for pharmaceutical and technology research. They have also been utilized in computer simulations to predict the pharmacokinetic, pharmacological, and toxicological behavior [11,12]. Currently, molecular docking is a highly successful and economical approach for the development and evaluation of drugs. This methodology produces data regarding the interactions between drugs and receptors, which can be utilized to forecast the positioning of potential drug candidates when they attach to their respective target protein [11,12].

In recent years, GC-MS has mostly been utilized to detect various bioactive therapeutic phytochemicals found in medicinal plants [13]. As far as we know, there are no existing reports on the GC-MS analysis of *P. thonningii*. There is currently no available information on the

antibacterial and docking research of *P. thonningii*. To address this research deficiency, we present a comprehensive analysis of the chemical composition, antibacterial properties, and docking studies of chloroform extracts derived from the leaves and seeds of *P. thonningii*.

MATERIALS AND METHODS

Sample Collection and Identification

The leaves and seeds of *P. thonningii* were collected from the vicinity of the College of Physical and Applied Sciences of Michael Okpara University of Agriculture, Umudike, on the 16th of January, 2016. The samples were authenticated at the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Sample Preparation

The samples were air-dried under a shade for thirty days and were ground into small particle sizes using an electric blender. The weights of the ground samples were 378 and 223 g for the leaves and seeds respectively. The samples were put in separate air-tight containers and labeled.

Sample Extraction Procedure

About 340 g of the ground leaf sample and 200 g of the ground seed sample of *P. thonningii* were weighed into separate bottles. 2.5 L of chloroform was introduced into the leaf sample while 1.5 L

of chloroform was introduced into the seed sample and allowed to stay for seventy-two hours. After that, the mixtures were filtered using Whatman No. 1 filter paper. The chloroform extracts were subjected to evaporation using a digital Heidolph Rotary Evaporator (4000 series) which was set at 45°C under reduced pressure of 0.01 mmHg to get the crude sample. After the evaporation of the chloroform, the extracts were allowed to stand for about 4 hours so that residual chloroform could evaporate completely to get the actual weight of the sample [14]. The extracts collected weighed 30.42 and 22.03 g for the leaf and seed respectively.

Quantitative Determination of Phytochemicals

The quantitative determination of phytochemicals such as alkaloids, flavonoids, phenols, saponins, tannins, terpenes and steroids in the leaf and seed extracts of *P. thonningii* was carried out following standard protocols. Alkaloids and saponins were determined by the method described by Harborne [15]. The method described by Nwoke *et al.* [16] was used in the determination of flavonoids while the Follins Dennis spectrophotometric method described by Kirk and Sayer [17] was used in the determination of tannins. The method reported by Indumathi *et al.* [18] was employed to determine terpenes while the method described by Okeke and Elekwa was used in the determination of steroids [19].

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The volatile phytochemical constituents of the extracts were determined using GC-MS analysis. The protocol employed here is as reported by Igwe and Abii [20]. GC analyses were carried out in SHIMADZU Japan gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25mm x 50m) and the conditions were as follows: Temperature programming from 80 – 200 °C for 1 minute, rate 5 °C/Min and at 200 °C for 20 minutes. FID temperature 300 °C, injection temperature 250 °C, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. GC-MS (Gas chromatography Mass spectroscopy) analysis was conducted using GCMS-QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 23 °C and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into a mass spectrophotometer with a detector voltage set at 1.5 kv and sampling rate of 0.2 seconds. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents used were all of analytical grade and were procured from Merck, Germany. The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature as well as using the database of National Institute of Standards and Technology (NIST).

Antibacterial Assay

The test organisms under study were clinical isolates obtained from Nnorex Laboratory, No. 85 St Michaels Road, Aba, Abia state, Nigeria. The isolates (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) were confirmed using the appropriate biochemical test. About 1.5 g of the chloroform extract of both the leaf and seed were separately dissolved in 10 ml of dimethyl sulfoxide (DMSO) (0.1%) to obtain a concentration of 150 mg/ml of the extract. The growth inhibition of the bacteria organisms by the extract was determined using the agar well diffusion method as described by Igwe and Echeme [21]. Mueller Hilton agar was the growth medium used for the microbes. The medium was prepared according to the manufacturer's instructions. The medium was sterilized for 20 minutes and was poured into sterilized Petri dishes and the dishes were allowed to cool and solidify. The 150 mg/ml obtained was further diluted to 75, 50, 25, 12.5 and 6.25 mg/ml. Sterile swab rod dipped into the appropriate test organism suspended in normal saline was used to uniformly streak the dried agar. Sterile cork borer of 5 mm in diameter was used to make wells on the medium. The wells were labeled with concentrations of the extracts. The various concentrations were dropped into each well to fullness using a sterile pasture pipette. Dimethyl sulfoxide (DMSO) solution was used as a control. The inoculated plates were then incubated at 37°C

for 24 hours after which the plates were observed for zones of inhibition of growth. A transparent plastic ruler was used in measuring the zone of inhibition and the results recorded in mm.

Determination of Minimum Inhibitory Concentration (MIC)

A tube dilution technique was used in the test. 1.5 g of the extract was dissolved in 10 ml of DMSO (0.1 %) to get 150 mg/ml of the stock solution, that was diluted using serial dilution by transferring 1 ml of the stock solution into 1 ml sterile nutrient broth to get 100 mg/ml solution that was further diluted to obtain other dilution: 75, 50, 25, 25 and 12.5 mg/ml. 0.5 ml of the standard test organism was inoculated into 0.5 ml of the diluted extracts and incubated at 37°C for 24 hours, using DMSO as a solvent and the test organism without extract was prepared and used as control. The turbidity of the solution on each tube was observed after 24 hours to find out if there was any growth. The lowest concentration of the extract dilution that showed no visible growth of the test organism was recorded as the MIC.

Preparation of Receptors and Prominent Compounds

The crystal structures of *S. aureus* (PDB ID: 1LMH), *E. Coli* (PDB ID: 1Q8I), *S. typhi* (PDB ID: 1TJY), *B. cereus* (PDB ID: 5V8E), and *P. aeruginosa* (PDB ID: 5WZE) were obtained from the RCSB Protein Databank. Water molecules and the cofactors were removed using the molecular molecular viewer software [22]. The PDBs of the most abundant compounds, Neophytadiene and 9,19-cyclolanostane-3,7-diol were downloaded from PubChem. They have been abbreviated as NPD and CLS, respectively.

In silico Studies

The compounds were docked to the receptors using the PyRx visual screening tool [23]. The macromolecules were uploaded, and the ligands were uploaded through Open Babel embedded in PyRx. The interactions of all docked complexes were visualized using Biovia Discovery Studio Visualizer [24].

RESULTS AND DISCUSSION

The results for the quantitative phytochemical screening of the chloroform leaf and seed extracts of *P. thonningii* are shown in Table 1.

Table 1: Quantitative phytochemical composition of *P. thonningii* leaf and seed extracts

Phytochemicals	Leaf extract (mg/kg)	Seed extract (mg/kg)
Alkaloids	15.5 ± 0.001	6.2 ± 0.01
Tannins	10.2 ± 0.01	4.2 ± 0.002
Saponins	9.6 ± 0.002	4.8 ± 0.011
Flavonoids	4.3 ± 0.03	2.5 ± 0.004
Terpenes	15.2 ± 0.001	15.9 ± 0.03
Steroids	16.9 ± 0.008	13.3 ± 0.001
Phenols	12.9 ± 0.01	7.9 ± 0.001

Data are mean ± standard deviation of triplicate determinations

Steroids have the highest concentration in the leaf extract followed by alkaloids, terpenes, phenols, tannins and then saponins. Flavonoids were observed to have the least concentration among the phytochemicals that are present in the leaves. Conversely, terpenes were seen to have the highest concentration in the seed extract followed by steroids, phenols, alkaloids, saponins, tannins and flavonoids. The alkaloid content of the leaves (15.5 mg/kg) is much higher than that of the seed (6.2 mg/kg). Most alkaloids are plant bases which exhibit certain physiological properties when used in herbal medicine. A lot of them have anti-malaria and antimicrobial activities [25]. The leaf also had higher tannins, saponins, flavonoids, steroids and phenols than the seed indicating a higher concentration of these phytochemicals. Steroids have been reported as a treatment remedy for cancer, arthritis, infertility and also in birth control [26]. The steroid content of the leaf

(16.9 mg/kg) and the seed (13.3 mg/kg) were high. This suggests the reason why the seeds and leaves are used in the treatment of infertility in men where increase in sperm count is important [10]. Also, steroids are higher in the leaf than the seed, which also implies that the leaf has a higher steroidal activity. The terpenes contents of the two extracts are high and insignificantly different. Terpenes have been reported to exhibit antibacterial, anti-tumor, anti-wrinkle, anti-oxidative, analgesic and immune-regulatory effects [27]. Saponins have been shown to possess antifungal properties and therefore help to protect the body from disease-causing organisms and infections [25]. The presence of tannins in plant has been associated with ulcer management, wound healing, control of bleeding and burns in herbal medicine. Phenolic compounds like flavonoids act as anti-inflammatory and antimicrobial agents [25].

Table 2: Volatile constituents from the GC/MS analysis of *P. thonningii* leaf extract

Peak No.	Phytochemical Components	Retention Time (min.)	Percentage Composition (%)
1	Neophytadiene	1.342	35.368
2	Dichloroacetic acid	5.323	0.492
3	Phytol	24.454	17.859
4	2-butoxy ethanol	24.681	1.352
5	Phytol acetate	24.896	3.330
6	n-Hexadecanoic acid	26.078	5.909
7	Ascorbic acid-2,6-dihexadecanoate	27.335	0.516
8	9,12-Octadecadienoic acid	27.859	7.637
9	L-Ascorbic acid,6-octadecanoate	28.010	1.080
10	Q	31.234	0.252
11	R	32.311	0.781
12	Nonacosane	33.865	3.943
13	Hentriacontane	35.757	5.561
14	Vitamin E	36.502	1.821
15	Triacontanal	37.602	4.892
16	Y- Sistosterol	39.540	5.330
17	Lup-20(29)-en-3-one	40.570	1.638
18	S	40.919	2.240

Q = (22S)-21-Acetoxy-6 α ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-3,20-dione

R = 1'-carboethoxy-1'-cyano-1,2-dihydro-3''H-cycloprop(1,2)-5-cholest-1-en-3-one

S = 3,11,18-triacetoxy-3,9- epoxypregnan-14-ol-20-one

Table 3: Volatile constituents from the GC/MS analysis of *P. thonningii* seed extract

Peak No.	Phytochemical Components	Retention Time (min.)	Percentage Composition (%)
1	Dichloroacetic acid	1.331	0.367
2	Trichloromethane	1.657	0.327
3	n-Hexadecanoic acid	25.996	0.750
4	9,12-Octadecadienoic acid	27.795	2.025
5	19-Cyclolanostane-3,7-diol	30.588	85.543
6	5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl chromone	32.137	0.646
7	Hentriacontane	35.594	0.862
8	Vitamin E	36.507	6.166
9	Stigmasterol	38.510	1.348
10	β -Sistosterol	39.441	1.964

Neophytadiene (Compound 1 in Table 2) is the most abundant volatile phytochemical detected in the GC-MS analysis of the leaf extract of *P. thonningii* with 35.368 % composition. Neophytadiene has been reported as a good analgesic, anti-pyretic, anti-inflammatory, antimicrobial and antioxidant compound [28]. Dichloroacetic acid was detected in both extracts. It has an active pharmacological display of anti-cancer and anti-neuropathic activities. It has been investigated as a treatment for post-ischemic recovery. It improves metabolism by NADH production stimulation, but may lead to a depletion of NADH in norxomia [29]. Phytol detected in the leaf extract is used to increase energy and to fight infection and are natural alternatives to use for hypertension, cancer tuberculosis [30]. n-Hexadecanoic acid (compound 6 in Table 2 and compound 3 in Table 3) has anti-inflammatory, antioxidant, hypocholesterolemic, pesticide, anti-andiogenic flavor, hemolytic, potent mosquito laviade [31]. 9,12-octadecadienoic acid found in both extracts has anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective and nematocidal properties [32]. L-Ascorbic acid-6-octadecanoate detected in the leaf extract as compound 9 has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties [33]. Nonacosane from the leaf extract has been reported to exhibit a strong antibacterial properties [34]. Hentriacontane detected in both extracts is used in diabetes

mellitus, bacterial infection, cancer, amoebiasis and leukemia. It is hypoglycemic, antibacterial, antioxidant, hepatoprotective, anticancer, antiestrogenic, and immunomodulant [35]. Vitamin E also present in both extracts possesses lower incidence of cardiovascular diseases, cancer, and dementias [36]. Phytol acetate detected in the leaf extract is a derivative of phytol which has been reported to possess antioxidant, anti-inflammatory, immune-modulating and antibacterial activities [30].

Stigmasterol was detected in the seed extract and have been reported to inhibit the development of various cancerous cells by inhibiting the promotion and growth of apoptosis of the cancer cells [37]. Triacontanal found in the leaf extract has shown the ability to alleviate negative effect of stress induced by salinity, toxicity, cold temperature, CO₂ and light deprivation [38]. Sistosterol was detected in both extracts. It could be considered for the development of potent anti-diabetic drugs [39]. Lup-20(29)-en-3-one was found in the leaf extract. It has a complex pharmacology displaying antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemo-preventive [40]. 3,11,18-triacetoxy-3,9-epoxypregnan-14-ol-20-one detected in the leaf extract has been reported to have antifungal property [41]. 19-Cyclolanostane-3,7-diol is the most abundant volatile phytochemical in the seed extract of *P. thonningii* with 85.543 %

composition and has been reported to have antibacterial properties [42].

Table 4: Zones of growth inhibition at varying concentrations of the leaf extract of *P. thonningii*

Test Organism	150 (mg/ml)	75 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)	6.25 (mg/ml)	DMSO (0.1%)
<i>S. aureus</i>	22.00±0.01	18.00±0.50	15.10±0.00	11.50±0.05	8.73±0.25	8.00±0.00	-
<i>B. cereus</i>	-	-	-	-	-	-	-
<i>E. coli</i>	18.50±0.00	17.00±0.00	14.10±0.25	6.03±0.01	-	-	-
<i>S. typhi</i>	15.10±0.05	13.05±0.00	6.40±0.00	-	-	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	-

Results are mean of triplicate determinations ± standard deviation; - = No zone of inhibition

Table 5: Zones of growth inhibition at varying concentrations of the seed extract of *P. thonningii*

Test Organism	150 (mg/ml)	75 (mg/ml)	50 (mg/ml)	25 (mg/ml)	12.5 (mg/ml)	6.25 (mg/ml)	DMSO (0.1%)
<i>S. aureus</i>	11.10±0.01	10.30±0.25	9.20±0.00	6.40±0.01	-	-	-
<i>B. cereus</i>	-	-	-	-	-	-	-
<i>E. coli</i>	10.00±0.50	8.25±0.00	-	-	-	-	-
<i>S. typhi</i>	-	-	-	-	-	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	-

Results are mean of triplicate determinations ± standard deviation; - = No zone of inhibition

Table 6: Minimum inhibitory concentrations of chloroform leaf and seed extracts of *P. thonningii*

Test microorganism	Leaf extract MIC (mg/ml)	Seed extract MIC (mg/ml)
<i>S. aureus</i>	25	50
<i>B. cereus</i>	-	-
<i>E. coli</i>	50	75
<i>S. typhi</i>	75	-
<i>P. aeruginosa</i>	-	-

From the results of the antimicrobial analyses, we can see interesting and promising results from Tables 4, 5 and 6 where two gram-negative and three gram-positive bacteria strains were used. It could be seen in Table 4 that *S. aureus* is the most susceptible to growth inhibition at the concentration of 150 mg/ml followed by *E. coli* and *S. typhi*, while *B. cereus* and *P. aeruginosa* were the most resistant strains at that concentration. At 75 and 50 mg/ml, *S. aureus*, *E.*

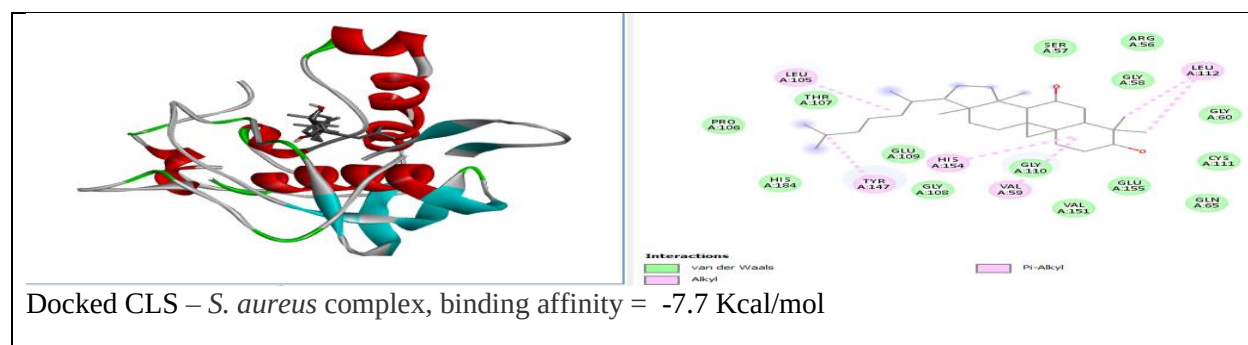
coli and *S. typhi* showed susceptibility to growth inhibition to the leaf extract respectively while *B. cereus* and *P. aeruginosa* were resistant to inhibition. At 25 mg/ml, *S. aureus* and *E. coli* showed susceptibility while *S. typhi* at this concentration was not able to show susceptibility. At 12.5 and 6.25 mg/ml, only *S. aureus* showed reasonable susceptibility while the other four bacteria organisms showed resistance to growth inhibition. It could be deduced at this juncture

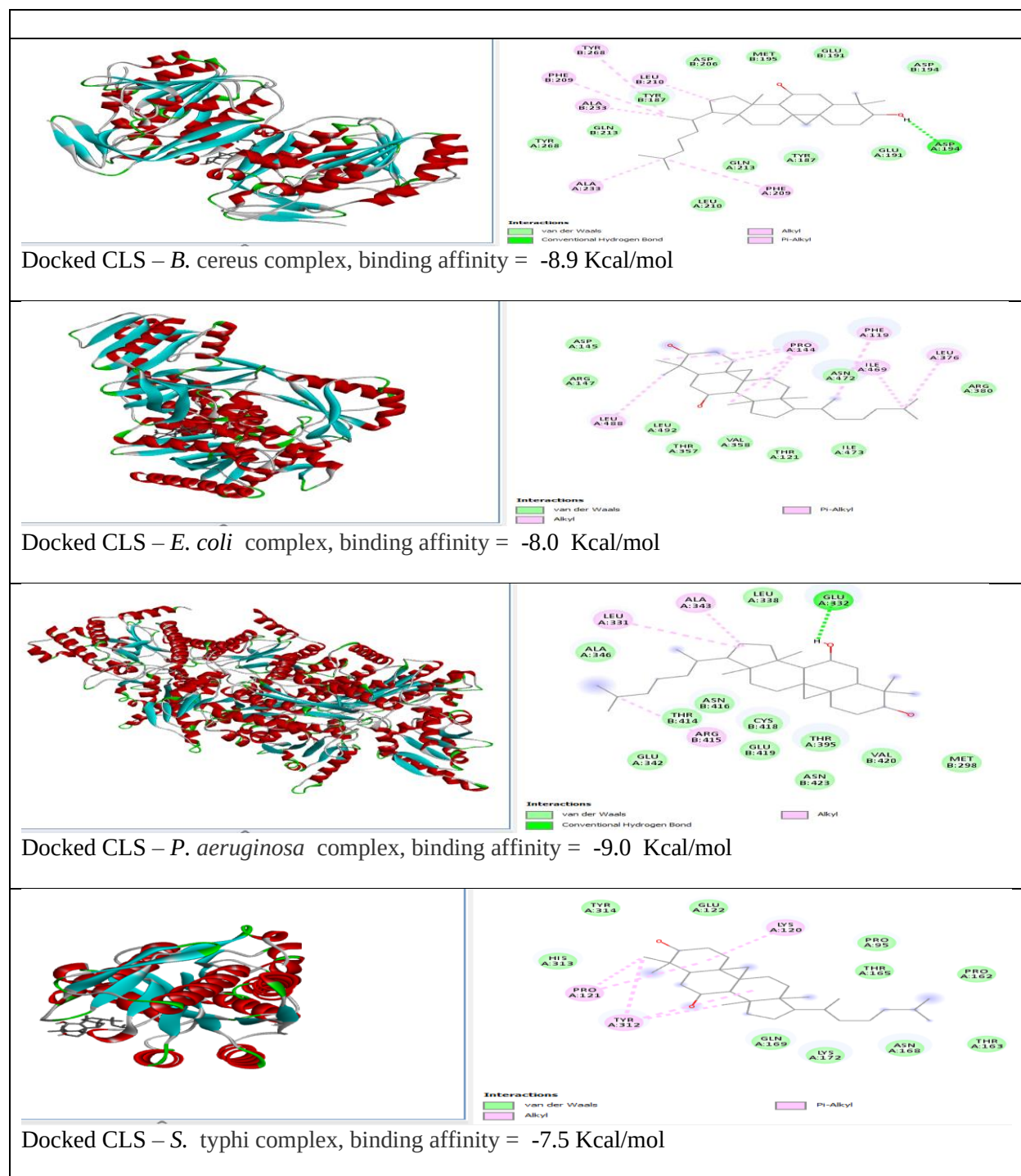
that there is a direct relationship between the concentration of the extract and susceptibility of the test organisms to growth inhibition.

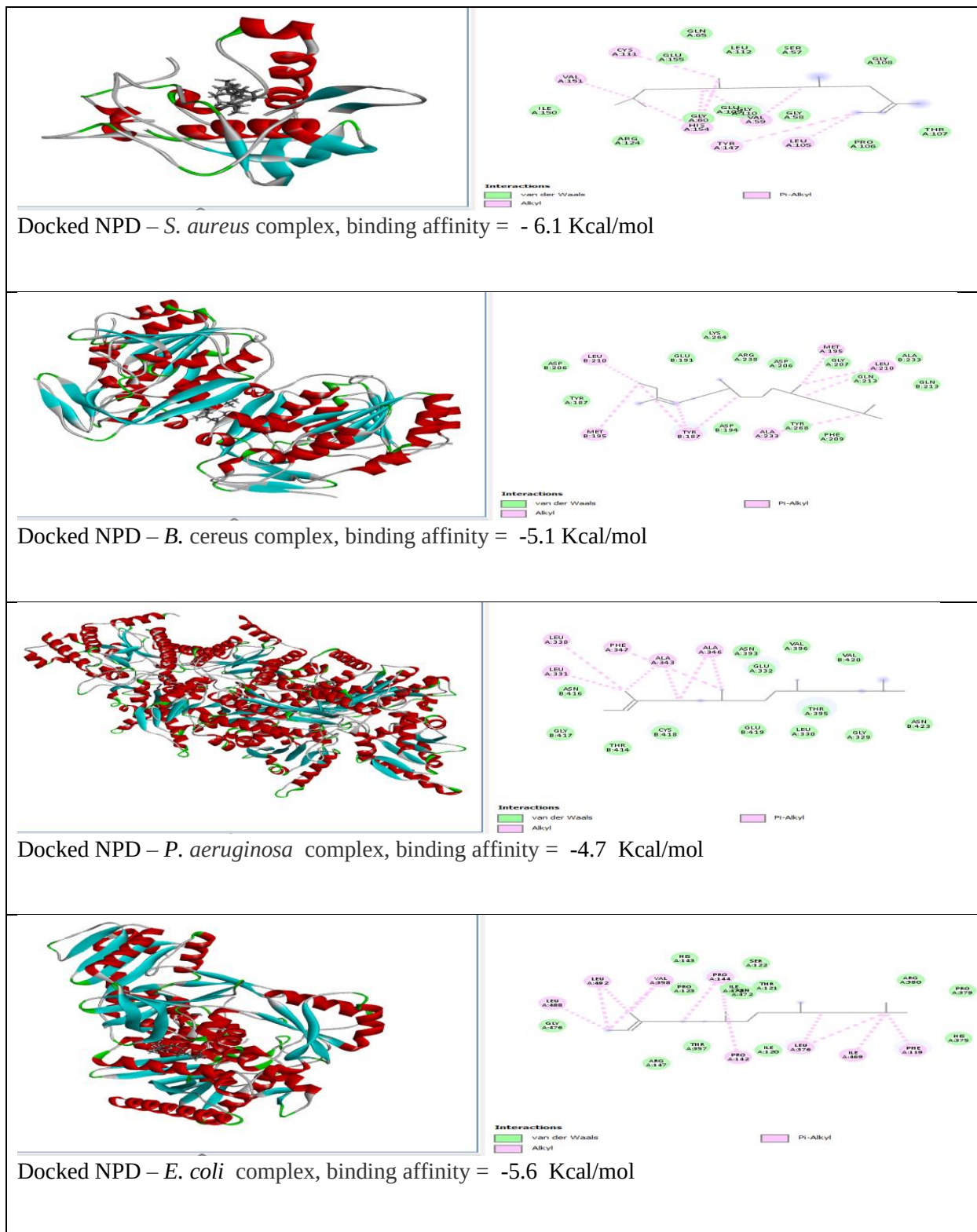
Also, in the seed extract as shown in Table 5, only one gram-positive bacterium (*S. aureus*) was able to show susceptibility at 150, 75, 50 and 25 mg/ml but showed resistance at 12.5 and 6.25 mg/ml. Moreover, one gram-negative bacterium (*E. coli*) showed susceptibility only at 150 and 75 mg/ml whereas it showed no susceptibility at 50, 25, 12.5 and 6.25 mg/ml. The remaining three bacteria organisms did not show any susceptibility at any of these concentrations. From Tables 4 and 5, the leaf extract showed a better promising antibacterial effect than the seed extract. The *S. typhi* showed susceptibility in the leaf extract at 150, 75 and 50 mg/ml while the same bacterium showed resistance in that of the seed extract. However, both extracts have shown antibacterial activity against some of the test organisms most probably due the presence of phytochemicals in them since certain phytochemicals have been reported to possess antibacterial activity [43]. This might be the reason why Ozolua *et al.* [44] reported that the

plant could be used for the treatment of bacterial infections. It was also found from literature that the leaves are used for the treatment of diarrhea, dysentery, worms and other intestinal problems caused by bacteria organisms [8,9,10].

From Table 6, the minimum inhibitory concentration (MIC) values of the leaf extract on *S. aureus*, *E. coli* and *S. typhi* are 25, 50 and 75 mg/ml respectively while that of *B. cereus* and *P. aeruginosa* show no minimum inhibitory concentration even at 150 mg/ml. This result shows that the chloroform extract of *P. thonningii* has an anti-microbial properties against *S. aureus*, *E. coli* and *S. typhi* but resistant to *B. cereus* and *P. aeruginosa*. Also, Table 6 shows that the minimum inhibitory concentration (MIC) values of the seed extract on *S. aureus* and *E. coli* are 50 and 75 mg/ml respectively, while *B. cereus*, *S. typhi* and *P. aeruginosa* have no minimum inhibitory concentration even at 150 mg/ml. The minimum inhibitory concentration is the lowest concentration of antimicrobial agent that prevents visible growth of a micro-organism. The docked complexes, 2D interactions and their binding energies are shown in Figure 1.







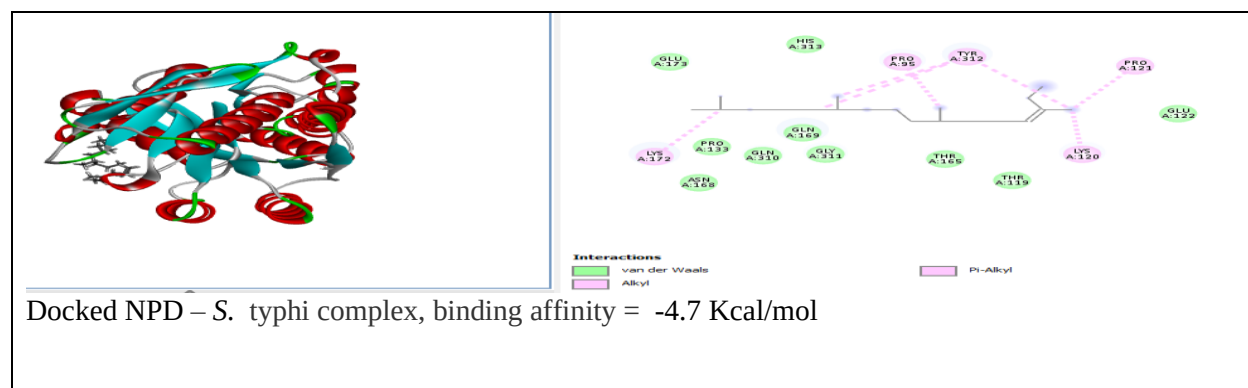


Figure 1: The docked complexes, 2D interactions and their binding energies

The negative binding affinities of the complexes predicted favourable docking scores. It indicated the possibility of the compounds to inhibit the bacteria species. Generally the CLS gave better docking score than NPD. This suggested that CLS may have better inhibitory activity than NPD. The CLS- *P. aeruginosa* docked complex gave the best pose of -9.0 Kcal/mol. This high docking score might have been influenced by a single hydrogen bond with the GLU 322A. H-bonding is a distinctive indicator of strong interactions between proteins and ligands, often leading to increased binding affinity [45]. During these interactions, the quantity of hydrogen bonds often enhances the inhibitory capacity towards the target. The ALA 343A, LEU 331A and ARG 415A residues exhibited alkyl/ π -alkyl interactions. In addition, it was discovered that van der Waals interactions occurred between CLS and the ALA 346A, ASN 416B, THR 414B, GLU 342A, MET298B, VAL 420B, ASN 423B, THR 395A, GLU 419 B and LYS 418B. Similar docking interaction have also been reported [11,12,46,47].

CONCLUSION

GC-MS technique gives us a better and easy avenue to analyze and identify some phytochemicals from plant samples. The phytochemical profiles of the leaf and seed chloroform extracts of *P. thonningii* have been investigated and proven to have medicinal properties. The use of *P. thonningii* in native herbal medications is supported by the ability of the extracts to inhibit the growth of some clinical isolates. The findings provide evidence for the traditional usage of this extract in herbal medicine and justify the need for additional research into its potential clinical uses. It is suggested that future study should prioritize investigating the fundamental mechanisms behind the plant's medicinal properties and undertaking clinical trials to confirm its effectiveness and safety in humans. Furthermore, it is necessary to conduct studies that investigate potential synergistic effects when combining conventional medications, as well as develop formulations that ensure optimal delivery.

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