Research Square

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Antimicrobial activity of Tetrapleura tetraptera (Uyayak) extracts, ADMET profiling and molecular docking of its bioactive compounds against dihydropteroate synthase of Escherichia coli

Glory P. Bebia
University of Calabar
Uwem Okon Edet (uwemedet27@gmail.com)
Arthur Jarvis University
Aniekan-Augusta Okon Eyo
University of Calabar
Agbor Yeneochia Ogar
Arthur Jarvis University
Joy Chinweokwu Ugwu
University of Calabar
Elizabeth Nkagafel Mbim
Arthur Jarvis University
Ofonime Mark Ogba
University of Calabar
Francisca O. Nwaokorie
University of Lagos

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Abstract

Background

Escherichia coli like other *Enterobactericeae* are frequent isolates implicated in food-borne diseases. The prevalence of multi-drug resistant *E. coli* isolates from street vended fruits and its salad is on the increase across Nigeria. This study was designed to evaluate the antimicrobial activity *Tetrapleura tetraptera* (Uyayak) against *E. coli* as well as the druggability and pharmacokinetics of its bioactive compounds using in-silico and in-vitro approaches.

Methods

We utilized previously reported standard protocols in the isolation, characterization and the identification of the isolates, and the collection and preparation of the *T. tetraptera* pods. Antimicrobial activity of the extracts was done using the Kirby-Bauer disc diffusion method. Resulting bioactive compounds from gas chromatography coupled to mass spectrophotometry (GC-MS) were converted into canonical stings and used to for target prediction in humans and ADMET properties using the SWISSADME and pkCSM tools. Bioactive compounds that met Lipinski's rule of five (ROF) were subjected to molecular docking against dihydropteroate synthase of *E. coli* using the AutoDock vina tool and the resulting interactions visualized in 2-D via Biovia Discovery Studio 21.

Results

The GC-MS analysis returned a total of twenty-eight (28) bioactive compounds. The abundance of the *E. coli* isolates varied according to location and fruit types. At trimethoprim and extracts concentrations of < 100mg/ml and < 100% respectively, the isolates showed resistance. A total of 13 bioactive compound showed zero violations to Lipinski's rule of five (ROF). ADMET analysis of the screened bioactive compounds showed favourable absorption (intestinal and water solubility) and toxicity (AMES and hepatoxicity) profiles than trimethoprim. Molecular docking revealed various amino residues interacting with dihydropteroate synthase and gave docking scores that ranged from − 4.0 to -5.3 kcal/mole for the bioactive compounds and − 6.5 5 kcal/mole for trimethoprim. Target prediction showed that all the bioactive are capable of reaching various targets with nuclear receptor being the most abundant target.

Conclusion

The bioactive compounds of *T. tetraptera* examined in this study showed favourable antimicrobial activity against *E. coli*, docking scores and pharmacokinetics, suggesting the need for further studies to validate their potential as antimetabolites for management of pathogenic *E. coli* infections.

Introduction

In Nigeria, there is a heightened awareness of the benefits of consuming fruits and vegetables [1]. They are a source of cheap and readily available micro and macronutrients, vitamins, minerals and phytochemicals [1]. These nutrients confer numerous health benefits to humans that includes growth maintenance, detoxification and prevention of diet-related diseases and deficiencies in humans [2][3]. Collectively, these benefits are essential for the overall wellbeing of a person [4]. In line with this awareness, there is an increase in the number of vendors that participate in street vending of fruits, fruits salads and vegetables [1]. Street vended fruits are sold either as whole or as ready-to-eat (RTE) or fresh cut fruits with the RTE fruits being the most popular form of consumed fresh fruits. The RTE fruits are very attractive and appealing to consumer because they are fresh, does not need further processing, affordable and suits the fast lifestyle of city dwellers [1][4][5].

Across Nigeria, atleast different fruit kinds are sold and they include pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*), orange (*Citrus sinensis*) and apple (*Malus domestica*). Others include cucumber (*Cucumis sativum*), cabbage (*Brassica oleracea var.* capitata), carrot (*Daucus carota*), berries, mangos, star apples, onion (*Allium cepa*), tomatoes (*Solanum lycopersicum*), lettuce (*Lactuca sativa*) and green-pepper (*Capsicum annuum*). Fruits and vegetables are also prone to microbial contamination as they are constantly in contact with the environment (soil, animals droppings used as manure, water and dust, insect bites, etc) and during harvest and post-harvest handlings [6][7]. Practices such as the use of untreated wastewater for irrigation and unhygienic post-harvest handling of the fruits and vegetables contribute to the contamination process. Other sources of contamination include the untreated water or water from dirty reservoir used in washing the fruits, transport equipment, crevices of blending machines, and unwashed hands and questionable hygiene of food handlers [7]. Among the RTE fruit handlers, microbial contaminants can also arise during the processing of the fruits (cutting, washing with water, packaging, ambience, from the normal flora of the handlers, etc). Studies have shown that RTE fruits could be a vehicle for the transmission of numerous microorganisms that include *Staphylococcus aureus, Bacillus cereus, Salmonella species, E. coli, Klebsiella spp*, and *Enterobacter faecalis* to mention a few [1][7][8][9]. Among these microbes, *E. coli* stands out as it can survive adverse environmental conditions via adhesion to surfaces and internalization to fresh products [10].

E. coli is one of the many pathogens that have been isolated from street vended fruits and vegetables [7]. *E. coli* is normal flora of the human large intestine and other warm-blooded animals [11]. Most strains are harmless, but some strains acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasion factors and become pathogenic [12]. These virulent strains are responsible for diarrheal infections worldwide, as well as neonatal meningitis, septicemia, and urinary tract infections (UTIs). The most common pathogenic strains of *E. coli* are the enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Shiga toxin–producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPIC) and Diffusely adherent *E. coli* (DAEC). Their virulence is attributed to virulent factors that they produce and these include colonization and adhesion factors, production of toxins and effectors [12] [13]. These pathogenic strains have been linked to various food-borne diseases outbreaks traceable to fruits and foods all over the world [13].

Furthermore, there are numerous reports of the multi-drug resistance among *E. coli* isolates from fruits and foods [11][14][15][16]. The incidence of infections caused by multidrug resistant strains *E. coli* is on the increase [15]. Added to this public health challenge is the fact that they have outstanding ability to act as a donor or recipient of resistant genes [15]. Commonly used antibiotics that are used to manage infections caused by species of the *Enterobacteriaceae* such as the extended-spectrum penicillins, cephalosporins, monobactams, carbapenems, fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamicin) are not spared the multidrug resistance feat of *E. coli* [1][16][17][18]. Multidrug resistant *E. coli* is on the WHO's list of priority and critical pathogens for which new antibiotics are urgently needed [19]. Member of the critical pathogens, especially the multidrug pathogens have been implicated in various hospital and nursing home infections [13][19].

Developing new antibiotics is a time consuming and financially demanding process [20]. To save time and cost, researchers have turned to several strategies and these include repurposing of existing approved drugs [21]. Yet, other have turned to screening bioactive compounds from medicinal plants [22][23]. In this study, the aim is to evaluate the antimicrobial activity of *T. Tetraptera* (Uyayak) pod that is widely used in cooking soups and in folk medicine to manage various ailments using in-vitro and in-silico approaches against *E. coli* isolates from street vended RTE fruits and salads. In addition, the pharmacokinetics of *T. Tetraptera* bioactive compounds and their molecular docking potentials against *E. coli* dihydropropate synthase (DHPS), a selective non-toxic drug target was also assessed.

Methods

Study location

The present study was carried out in Cross River State (section 1A in Fig. 1). Cross River State is located in southern Nigeria. Administratively and geopolitically, it is regarded as one of the nine Niger Delta States of Nigeria. It had a total of 18 local government areas (Section 1A). Sampling for fruits and fruit salads was carried out in Calabar Municipality and Calabar South Local Government Areas, both regarded as the capital of Cross River State. The capital as seen in the map is located in the southern part of the State and lies on latitudes 4˚50'N and 5˚10'N and longitudes 8˚17'E and 8˚20'E. It is bound to the north and east by Odukpani and Akpabuyo Local Government Areas of the State, respectively. The population of the State capital has increased from 82,100 in 1975 to 375,196 in 2006 [24]. The State has an abundance or rainforest [24]. With a rich middle population that is on the increase, there is an increased consumption of fruits and fruit salads [25][26]. From both LGAs, fruit and fruit Salad vendors abound in major markets and were identified for sampling.

Collection of fruit, fruit salad and T. tetraptera samples

From all four markets, various fruits and fruit salads were obtained. The sampled markets were Bogobiri, Watt, Akim and Marian. Collected fruits from each market were watermelon, pineapple, oranges and apples in addition to fruit salads. A total of 15 samples were collected from each location. Collection of fruits was done aseptically using Ziplog bags. Collected samples were placed in ice and transported to the laboratory within 2 hours of collection for microbiological analysis. *T. tetraptera* (Uyayak) pod was purchased from Marian and Watt Markets, respectively in August 2022 and also stored in Ziplog bag (Fig. 2). On the hand, the *T. tetraptera* pods were transported without ice within one hour of collection.

Preparation of plant extracts

The *T. tetraptera* pods were dried milled using a sterilized stainless locally fabricated kitchen grater. The milled pellets were weighed (20 g) and soaked separately in distilled water and analytical grade ethanol. The mixtures were allowed overnight with the conical flasks holding each mixture wrapped with aluminium foil. After overnight soaking of the pellet in the solvents, the mixtures were filtered using Whatman no. I filter paper and then heated using a water bath operated at 60^{O}C until a slurry was obtained. The resulting slurries were stored in sterilized Bijou bottles for further analysis. All the protocols were done as previously reported [28][29].

GC-MS analysis of the T. tetraptera

This was done as reported previously [28]. Exactly 10 g of the milled *T. tetraptera pod* was added to 20 ml of methanol and the resulting mixture placed on an orbital shaker for 15 mins and the mixture allowed to stand for another 15 mins after shaking. The mixture was then filtered and the filtrate concentrated to 5 ml using a rotatory evaporator. From the concentrate, bioactive compounds were screened using an Agilent 5890N gas chromatography fitted with an autosampler connected to an Agilent mass spectrophotometric detector. All the operating conditions were as previously reported. The bioactive compounds in the sample were identified and interpreted using the database of National Institute Standard and technique (NIST) [28].

Microbiological analysis

Collected fruit and fruit salads were allowed to assume the temperature of the laboratory before being used for microbiological analysis. The samples were first weighed out (100 g each) for each fruit type and the fruit salad. The samples were then placed in sterile stomacher bags one per fruit type for each location and homogenized using a Biobase Sterile Homogenizer (Model Bk-Shg04, China) [30]. The homogenate was then serially diluted using a ten-fold serial dilution [31]. The content of the third and fourth dilutions were emptied into test tubes holding freshly prepared *E. coli* (EC) broth medium that was supplemented with 0.05 mg/l cefixime to enrich *E. coli.* The test tubes were then incubated overnight and after overnight incubation, pour plating was carried out using 1 ml aliquot using of the inoculum onto MacConkey agar and Eosin Methylene Blue agar plates. Resulting colonies were examined for the distinctive morphology of *E. coli* and characterized using various routine biochemical tests [32]. Characterized isolates were then stored in nutrient agar slants for antimicrobial assay.

Antibacterial susceptibility of the crude extract

A total of forty (40) *E. coli* isolates were subjected to antimicrobial sensitivity test using the crude extracts and trimethoprim as control. This was done using the agar-well diffusion technique [33]. The stored isolates were first sub-cultured using freshly prepared nutrient agar and incubated overnight at 37 ^{o}C. The isolates were then suspended each in sterilized 5 ml peptone water and the concentration of the isolates adjusted to MacFarland standard which holds approximately 10^{7} cells/ml. From each standardized inoculum, 1 ml was plated out using surface plating technique on freshly prepared Mueller-Hinton agar and incubated for 1 h. After incubation, holes were bored aseptically as previously reported [23] and various concentrations of the extracts (100, 50 25 and 12.5%) and trimethoprim (100, 50, 25 and 12.50 mg/l) freshly prepared were dispensed in triplicates per plate and incubated at 37 ^{o}C overnight. The resulting zones were measured and interpreted as sensitive, intermediate and resistant as previously reported [34].

Prediction of ADMET properties of the ligands

Bioactive compounds from the various ligands were screened for their Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties using the pkCSM prediction tool. The pkCSM tool is a well-established tool for the prediction of ADMET properties of lead drug compounds [35]. First, the ligands were converted into their respective SMILE strings using PubChem. The strings were then used to individually to predict the ADMET properties of the various ligands together with their molecular descriptors.

Lipinski rule of five (ROF) screening

The ligands (bioactive) compounds obtained from the GC-MS analysis were further screened for Lipinski's ROF using the SWISSADME tool. The SWISSADME is a free-web tool used in the prediction of lead compounds or small molecules pharmacokinetics, drug-likeness and medicinal chemistry friendliness [36]. Following ADME screening, ligands that meet the ROF were further prepared for molecular docking.

Ligand preparation for docking

The screened ligands were prepared for molecular docking using various tool. First the structures of the ligands were retrieved from their unique SMILES strings using Chem3d 15.1 tool. Following retrieval of their structures, the energies of the ligands were minimized and saved in in pdb files. The ligands were further prepared using AutoDock vina 4.2 and saved in pdbqt files individually [37].

Preparation of protein for molecular docking

From the Research Collaboratory for Structural Bioinformatics (RCSC) protein database, the dihydropteroate synthase of *E. coli* was retrieved. Other details of the protein as obtained from MCULE tool and RCSC PDB were 1AJ2, DHPS_ECOLI, P0AC13 and 83333 for the PDB ID, Uniprot name, Uniprot Accession ID and Uniprot taxonomic ID. As a protein complexed with native ligands (n = 2), the binding or active site of the protein was retrieved using these native ligands as guide. Definition of the binding site was done using Biovia Discovery Studio Client 21. The binding site was defined to be x = 41.244328, y = 4.789960, z = 8.014888 with a radius of 15.434639. The protein was further prepared by removing water molecules and the resulting structure saved in pdb format. The protein in pdb was converted into pdbqt file using the AutoDock vina 4.2 for docking [37].

Molecular Docking And Visualization

Molecular docking was performed the AutoDock vina 4.2 [37] First the various AutoDock files were retrieved and configured accordingly to align with the various prepared ligands and protein. Using the docking command lines, docking was performed and the resulting docking modes split for each ligand. The various interactions in the various binding modes were then visualized in 2D using the Biovia Discovery Studio Client 21 software to display the amino acids, types of bonding and bond lengths.

Results

Figure 3 shows the distribution of the *E. coli* isolates according to fruit type. A total of 40 isolates were obtained and according to fruit type, water melon with 16 (40%) isolates had the highest occurrence of *E. coli* followed by fruit salad with 11 (27.5%). The distribution of the isolates from other types of fruits was 2 (5%), 5 (12.5%) and 6 (12.5%) for pineapple, orange and apple, respectively. Table 1 shows the distribution of the *E. coli* isolates according to locations they were purchased.

From Table 1, the highest number of *E. coli* isolates was observed from Bogobiri with a total of 19 (47.5%) followed by Watt with 9 (22.5%) isolates. The location with the least amount of *E. coli* was Akim with 7 (17.5%) isolates.

Table 1
Distribution of the *E. coli* isolates according to location

Location	Fruit type					Total (%)
	Watermelon	Pineapple	Oranges	Apple	Fruit Salad	
Akim	2	-	-	1	4	7 (17.5)
Bogobiri	10	2	3	2	2	19 (47.5)
Marian	2	-	-	1	2	5 (12.5)
Watt	2	-	3	1	3	9 (22.5)
Total	16	2	6	5	11	40 (100.0)

Figure 4 shows the GC-MS chromatogram (abundance versus retention time) of the bioactive compounds from *T. tetraptera*. The bioactive compounds are represented by the various peaks. The peaks were 26, representing a total of 26 bioactive compounds. Table 2. The table shows the retention time (min) of the 28 bioactive compounds, their molecular weights, peak areas (%) and development (min). The retention time ranged from 8.274 to 24.710 mins for compounds methone and beta-Caryophyllene. On the other hand, the molecular weights of the various compounds ranged from 16.04 to 478.7 g/mole for methone and carpaine, respectively. Peak area shows that lupeol was the most abundant with a peak area of 21.71 while eugenol had the least peak area with a value of 1.13.

Table 2
GC-MS output of the *T. tetraptera*

Compounds	R.T (min)	MW (g/mol)	Peak area (%)	Dev (min)
Methone	8.274	16.04	7.86	36
Lemonene	8.839	136.24	4.21	51
Octanol	9.421	130.228	2.3	74
Methanol	9.718	156.27	9.42	62
Dihydrocarvone	10.352	152.23	2.74	42
Carvone	10.894	150.22	1.82	69
Beta-bourbonene	12.251	204.35	8.15	19
Methyl-10-methylheptadecanoate	12.758	298.00	1.64	52
Alpha-copaene	12.962	204.00	3.71	63
Campesterol	14.313	401.01	2.53	28
Piperitone	14.802	152.23	5.49	39
Beta-pinene	15.428	136.23	7.82	47
Lupenon	15.820	424.00	2.64	51
Beta amyrene	16.326	410.00	1.22	58
Terpinen-4-ol	16.711	154.25	3.43	74
Lupeol	18.336	426.00	21.71	39
3-Carene	18.758	136.24	1.62	42
Butanedioly dihydrazide	20.213	112.00	2.44	72
Alpha-pinene	20.801	136.23	8.79	68
Eugenol	21.369	164.2	1.13	53
Sabinene	21.741	136.23	6.57	31
15-Hydroxypentadecanoic	22.303	258.38	3.86	29
Carpaine	22.718	478.7	4.14	72
T-Cadinol	23.352	222.27	2.72	54
Alpha-ocimene	23.694	136.24	6.63	18
Olean-12-en-3-0ne	23.857	424.00	1.77	24
n-Hexadecanoic acid	24.293	256.00	3.42	36
Beta-caryophyllene	24.710	204.36	0.86	41

Table 3 shows the summary of the antimicrobial sensitivity profile of the aqueous and ethanolic extracts of *T. tetraptera*. Among the isolates that were sensitive, ethanolic extract was better than aqueous extract at all concentrations used. At 100%, 38 and 28 isolates, representing 95% and 70.0% were sensitive while at 50% of the extract concentration, 34 and 19 isolates were sensitive, representing 85.0% and 47.5% of the total isolates for ethanolic and aqueous extracts. As the concentration decreased, the number of isolates that were sensitive decreased while those that showed resistance increased. At concentrations of 6.25 and 3.125%, the number of isolates that were resistant to the aqueous and ethanolic extracts were 34 and 17 isolates, respectively. Comparatively, trimethoprim showed better results than the extracts used at the all concentrations (100 mg/l to 3.125 mg/l) used in this study.

Table 3
Antimicrobial sensitivity profile of the extracts and trimethoprim

Extracts and control	100 (%)	50 (%)	25 (%)	12.5 (%)	6.25 (%)	3.125 (%)
Sensitive						
Aqueous extract	28 (70.0)	19 (47.5)	14 (35.0)	11 (27.5)	8 (20.0)	4 (10.0)
Ethanolic extract	38 (95.0)	34 (85.0)	28 (70.0)	27 (67.5)	20 (50.0)	19 (47.5)
Trimethoprim (mg/l)	40 (100.0)	39 (97.5)	38 (95.0)	33 (82.5)	29 (72.5)	17 (42.5)
Resistant						
Aqueous extract	10 (25.0)	16 (40.0)	24 (60.0)	26 (65.0)	31(77.5)	34 (85.0)
Ethanolic extract	2 (5.0)	6 (15.0)	10 (25.0)	12 (30.0)	18 (45.0)	17 (42.5)
Trimethoprim (mg/l)	0 (0.0)	1 (2.5)	2 (5.0)	6 (15.0)	10 (25.0)	20 (50.0)
Intermediate						
Aqueous extract	2 (5.0)	5 (12.5)	2 (5.0)	3 (7.5)	1 (2.5)	2 (5.0)
Ethanolic extract	0 (0.0)	0 (0.0)	2 (5.0)	1 (2.5)	2 (5.0)	4 (10.0)
Trimethoprim (mg/l)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.5)	1 (2.5)	3 (7.5)

Key: ≤10 mm = Resistant, 11–15 mm = Intermediate and ≥ 16 mm = Sensitive.

Table 4 shows the predicted ADMET properties for ligands that meet Lipinski ROF. Adsorption was evaluated using water solubility, intestinal absorption, skin permeability, P-glycoprotein substrate, P-glycoprotein I and II inhibitors. Water solubility values of the study bioactive compounds ranged from − 2.25 to 5.477 while that of trimethoprim was − 2.744 and it was within the range of our study ligands. Intestinal absorption for the various ligands ranged from 91.485 to 95.257 and was higher than that of trimethoprim which was 71.787. Skin permeability for the ligands ranged from − 1.063 to -2.715 and − 2.735 for trimethoprim. A total of 11 ligands were not substrates P-glycoprotein while ligands II and X as well trimethoprim were substrates. All the ligands as well trimethoprim were not inhibitors P-glycoprotein I and II inhibitors. Distribution was evaluated using VDss (human), fraction unbound (human), BBB and CNS permeabilities. From the predicted result presented in Table 4, the VDss values ranged from − 0.574 to 0.812 for the ligands and 0.954 for the control drug. Fraction unbound values ranged from 0.0104 to 0.514 for the ligands and 0.554 for the control drug. For BBB and CNS permeabilities, the values for the ligands ranged from 0.084 to 0.792 and − 2.977 to -1.763, respectively while for trimethoprim, the respective values were − 1.225 and − 3.273 for BBB and CNS permeabilities. The predicted parameters for metabolism were CYP2D6 and CYP3A4 substrates and CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibitors. From the predicted result presented in Table 4, none of the ligands as well as trimethoprim were substrates to CYP2D6 while to CYP3A4, 11 ligands were not its substrates but ligands 10, 13 and trimethoprim were substrates. All the ligands as well as the trimethoprim were not inhibitors to CYP2C19, CYP2C9, CYP2D6 and CYP3A4; however, ligand VIII was predicted to inhibit CYP1A2. The predicted excretion parameter showed that total clearance ranged from 0.191 to 1.86 for the ligands while it was 0.98 for trimethoprim. All the ligands and the control drugs were not substrates to renal OCT2. Ames's toxicity profiling of the ligands and trimethoprim showed that all the ligands apart from VIII and trimethoprim could be carcinogenic. On the other hand, all the ligands did not indicate hepatoxicity concerns while trimethoprim was predicted to be hepatoxic. Furthermore, all the ligands as well as trimethoprim were not inhibitors to hERG I and II.

Figures 5a and 5b show the summary of the various predicted targets for the various ligands and trimethoprim. The result showed various targets that ranged from 6 to 10 targets for the various bioactive compounds while for trimethoprim, the number of targets were 7. Figure 6 shows a summary of the top predicted targets for the various ligands and trimethoprim. The top targets were family A G protein coupled receptors, fatty acid binding family, oxidoreductase, enzyme and lyase for the ligands while for trimethoprim, it was kinase. The most frequent target was nuclear receptor that was the most frequent target in six of the 13 bioactive compounds.

Table 4
Predicted ADMET properties for ligands that meet Lipinski ROF

ADMET	Predicted values													
Absorption	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Water solubility (log mol/L)	-2.668	-3.568	-2.556	-2.217	-2.385	-2.586	-2.296	-2.25	-4.31	-4.724	-4.073	-4.472	-5.477	-2.74
Caco2 permeability (log Papp in 10^{-6} cm/s)	1.229	1.401	1.471	1.376	1.412	1.134	1.502	1.559	1.438	0.849	1.479	1.411	1.565	0.03
Intestinal absorption (human)	97.324	95.898	92.552	95.257	97.555	97.957	94.014	92.041	91.485	91.891	94.296	95.155	92.51	71.7
Skin Permeability (log Kp)	-1.872	-1.721	-1.531	-1.919	-2.131	-1.915	-2.182	-2.207	-2.714	-2.782	-1.923	-1.063	-2.715	-2.73
P-glycoprotein substrate	No	Yes	No	No	No	No	No	No	No	Yes	No	No	No	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Distribution														
VDss (human) (log L/kg)	0.174	0.396	0.183	0.137	0.182	0.176	0.21	0.24	-0.771	0.812	0.42	0.364	-0.574	0.95
Fraction unbound (human)	0.458	0.48	0.488	0.453	0.526	0.47	0.514	0.251	0.237	0.378	0.28	0.389	0.104	0.55
BBB permeability	0.607	0.732	0.648	0.584	0.585	0.6	0.563	0.374	-0.318	-0.351	0.596	0.792	-0.084	-1.22
CNS permeability	-2.155	-2.37	-2.174	-2.119	-2.478	-2.169	-2.473	-2.007	-2.977	-2.948	-2.151	-1.912	-1.763	-3.27
Metabolism														
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No	No	No	Yes	No	No	Yes	No
CYP1A2 inhibitior	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No
CYP2C19 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Excretion														
Total Clearance	0.244	0.213	1.558	1.182	0.273	0.191	1.269	0.282	1.752	0.856	1.085	0.462	1.86	0.98
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity														
AMES toxicity	No	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes
Max. tolerated dose (human)	0.821	0.777	0.723	0.94	0.773	0.843	0.857	1.024	-0.922	-0.685	0.343	0.736	-0.713	0.01
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No

ADMET	Predicted values													
hERG II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Oral Rat Acute Toxicity (LD50)	1.796	1.88	1.679	1.946	1.87	1.777	1.811	2.118	1.69	2.968	1.918	1.702	1.449	2.93
Oral Rat Chronic Toxicity (LOAEL)	2.028	2.336	1.981	2.017	1.98	2.068	2.02	2.049	2.939	-1.167	1.475	2.414	3.109	1.13
Hepatotoxicity	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes
Skin Sensitisation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No
T.Pyriformis toxicity	0.42	0.579	0.207	0.333	0.419	0.439	0.189	0.3	0.907	0.285	1.49	0.924	0.865	0.30
Minnow toxicity	1.205	1.203	1.193	1.29	1.402	1.23	1.545	1.702	0.085	1.127	0.743	0.676	-0.956	2.49

Key: I = Methone, II = Lemonene, III = Octanol, IV = Menthol, V = Dihydrocarvone, VI = Piperitone, VII = Terpinene-4-ol, VIII = Eugenol, IX = 15-Hydroxypentadecanoic acid, X = Carpaine, XI = T-Cadinol, XII = Alpha-ocimene and XIII = hexadecanoic acid.

Figure 7 shows the various interactions between the various ligands and trimethoprim against of dihydropteroate synthase of *E. coli*. The various docking poses shows the various bond lengths and the amino acid residues that were involved in the interactions between the ligands and the enzymes. Table 5 shows the various amino acids involved in the interactions and their docking scores. The amino acid residues and their various docking scores and values ranged from − 4.0 to -5.3 kcal/mole and this was lower than that of trimethoprim that returned a docking score of -6.5 kcal/mole. However, one of the ligands, carpaine returned a positive docking score of + 44.3 kcal/mole, indicating an unfavorable docking. The interacting amino acid residues varied from one ligand to the other. As shown in Table 5, the most common amino acid residue among the bioactive compounds was isoleucine 117 followed by threonine 62 as both residues were found to be involved in eight and six complexes, respectively. The amino acids also varied in terms of number. Trimethoprim complexed with 9 amino acids and this was the highest while the bioactive compounds or test ligands complexed with 2 to 7 amino acid residues. However, carpaine complexed with a total of 8 amino acids.

Table 5
Amino residues involved in binding of the various bioactive compounds against dihydropteroate synthase

Bioactive compounds	Amino acid residues	Docking/Vina scores (kcal/mole)
1	Lys 221 and Ile 117	-4.8
2	Arg63 and Ile 117	-4.4
3	Gly217, Phe190, Ile117 and Asp 185	-4.0
4	Asn22 and Ile117	-4.5
5	Arg225, Thr62 and Met 139	-4.7
6	Thr 62, Ile117 and Met 139	-4.8
7	Thr62, His257 and Arg63	-4.8
8	Thr 62, Arg 225, Ile117, Phe190 and Lys221	-5.1
9	Thr62, Asn115	-5.3
10	Ser61, Lys221, Asn22, Met139, Asp105, Asn115, Ile117 and Asp96	44.3
11	Lys221 and Phe190	-4.4
12	His257, Ile20, Met139 and Phe190	-4.4
13	Thr62, Met139, Ile117, Phe190, Lys221, Arg63 and His63	-4.8
Trimethoprim (2,4-diamino-5-(3',4',5'-trimethoxybenzyl) pyrimidine)	Asn22, Asp96, Met139, Arg225, Glu 60, Ile20, His257, Lys221 and Asn 115.	-6.5
Key: I = Methone, II = Lemonene, III = Octanol, IV = Menthol, V = Dihydrocarvone, VI = Piperitone, VII = terpinene-4-ol, VIII = eugenol, IX = 15-hydroxypentadecanoic acid, X = carpaine, XI = T-cadinol, XII = alpha-ocimene and XIII = hexadecanoic acid and XIV = Trimethoprim		

Discussion

The study was aimed at evaluating the antimicrobial property of *T. tetraptera* against *E. coli* from street vended RTE fruits and salads using in-vitro and in-silico approaches. The prevalence of the *E. coli* isolates obtained in this study showed differences in terms of fruit type and location. Water melon returned the

highest abundance of *E. coli* while in terms of location, Bogobiri had the highest number of *E. coli* isolates. The location is one of the most densely populated areas in Calabar Cross River State as it is home to a thriving community of several ethnic groups, albeit dominated by Muslims that specialize in selling different kinds of barbeques (cattle, ram, chicken, etc). The bacterium *E. coli* is a well-known commensal and normal flora of the lower intestines of warm-blooded animals such as cattle [11]. Its isolation from various ready-to-eat fruits and fruit salads as obtained in this study corroborates earlier reports [11][38] [10] and is a great public health concern [13]. Although they are commensals, some strains notably the enterotoxigenic, enterohemorrhagic, enteropathogenic, enteroinvasive and enteroaggregative *E. coli* are pathogenic [11] and have the capacity to infect humans and trigger outbreaks [13][39]. The isolation of *E. coli* in various fruits samples affirms the fact that fresh fruits could be an important vehicle for the spread of *E. coli* including the pathogenic strains and other Enterobacteriaceae [38][39][40][41]. The presence of pathogens in food and fruits is largely due to poor hygiene practices that is usually observed by these fruit vendors [40]. As a rule of thumb, majority of the fruits' vendors including the ones sampled in this study sell their fruits in the open where dust, flies and other vectors or vehicles are difficult to control. Added to this is the fact that these vendors always sell their fruits in front of open drainage or gutters that are receive indiscriminately all manner of wastes and this was the case in all the study locations. Although we did not profile them for pathogenic strains, the *E. coli* isolates obtained in this study showed resistance to trimethoprim and the extracts used in this study even though this was concentration dependent.

The main concern of pathogens such as *E. coli* being transmitted in foods including fruits has been that of food-borne disease outbreak [19]. In line with this concern, numerous reports of outbreaks across the globe exists [13][10] where they have been linked to some indicator illnesses such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome [10]. Another concern that is on the increase is that of the spread of antibiotics resistance genes to one another [11]. *E. coli* isolates in this study showed resistance to the standard drug at concentrations that ranged from 50 mg/ml to 3.125 mg/l. This corroborates an earlier study that showed 83.3% (n = 76) of *E. coli* isolates were multi-drug resistant and harboured several virulence genes that included *aggr*, *eae* and *elt* [11]. Also worrisome is the ability of the bacterium to survive various environmental conditions that include adhesion to surfaces and internalization in fresh products [10] and spread of resistant genes. Resistant isolates of *E. coli* have been shown to harbour resistant genes to various antibiotics that includes the tetracycline, sulfonamides, streptomycin and apramycin [14] and are linked to poor clinical outcomes [42]. Widespread resistance amongst pathogens have promoted the search of safer and more efficacious antibiotics, especially of medicinal plant origin [22][23][28][29].

T. tetraptera was examined for its antimicrobial property against *E. coli* using in-vitro and in-silico approaches. *T. tetraptera* pod is edible and it is commonly used in cooking as spices in several local cuisines and its medicinal properties [29][17][43]. The extracts both aqueous and ethanolic showed antimicrobial activity in a concentration dependent fashion and was comparable to that of trimethoprim at the highest concentration of 100 mg/ml. The antimicrobial activity reported here is in line with a previous study that reported zones that ranged from 12.00 to 21.30 mm against *E. coli* isolates [29]. In addition to the antimicrobial activity of the extracts, its bioactive compounds were also examined using GC-MS. The result revealed a total of twenty-eight (28) bioactive compounds out of which only 13 meet Lipinski's ROF without any violation. According to the Lipinski's ROF, an orally active drug should not violate more than one of its criteria (MW < 500; ≤ 5 H bond donor; ≥ 10 H bond acceptors, and log *P* less than 5) [44]. This implies that all 13 bioactive compounds are potential oral drug candidates.

In addition to the Lipinski's ROF, we also evaluated their ADMET properties. ADMET properties are important properties that must be taken into consideration during drug discovery process [45]. Absorption of a drug is very important consideration in drug discovery, especially for drugs intended for oral consumption [46]. The water solubility of the ligands was higher than that of trimethoprim. Similarly, the intestinal absorption of the bioactive compounds ranged from 91 to 95% compared to that of trimethoprim that was 71.7%. The ligands further showed variance for the evaluated distribution properties. The result of the BBB permeation indicates that all the ligands could cross the BBB compared to trimethoprim which is predicted to poorly cross the BBB [47]. Drug efficacy or the efficacy of a lead compound depends a lot on its distribution in various tissues and explains the lack of relationship between plasma levels and their end biological effects [48]. The metabolism of the ligands showed some consistencies as shown by the various cytochrome systems in the liver through various cytochromes systems. None of the ligands including trimethoprim were substrates to CYP2D6 while 2 out 13 ligands were substrates to CYP3A4, implying that the majority will not be metabolized easily. However, the bulk of ligands were not inhibitors to the various cytochrome inhibitors including CYP3A4, that are known to clear certain drugs from the system [49] further affecting their bioavailabilities. Toxicity profiling of the ligands indicate cancer risk as revealed by a positive Ames's test for ligand 8 only [50] and an absence of hepatoxicity for all the lead compounds except trimethoprim. In addition to the ADMET properties, the ligands were docked against dihydropteroate synthase of *E. coli*.

Molecular docking has emerged as the most utilized in-silico or computational approach for studying interactions between proteins and other macromolecules with small lead drug compounds [51] [22][23]. We docked a total of 13 ligands and trimethoprim against dihydropteroate synthase of *E. coli* and the docking scores for the various ligands ranged from − 4.0 to -5.3(kcal/mole while trimethoprim gave a docking score of -6.5 kcal/mole. The higher docking score shown by trimethoprim indicates a better binding with dihydropteroate synthase even though it showed poor absorption and toxicity profiles. The scores shown by the ligands in our study is similar to those obtained in an earlier study with bioactive compounds from honey that were docked against dihydropteroate synthase of *Staphylococcus aureus*. The choice of dihydropteroate synthase is predicated on the fact only prokaryotes such as bacteria elaborate this important enzyme that is involved in folic acid synthesis while eukaryotes do not and have to rely on an external supply of folic acid since they lack this enzyme, making an important drug target [52]. For a drug to be effective, it has to be able to get to its target. In this case, the target dihydropteroate synthase is an enzyme that belongs to the ligases class of enzymes. From the predicted targets, all the ligands had enzymes as one of their predicted targets. The bioactive compounds despite their lower docking scores compared to the trimethoprim showed better pharmacokinetics, especially in terms of absorption and toxicity profiles.

Limitations Of The Study

The study had several limitations. First, the number of fruit types examined was small. Second, the *E. coli* isolates were not identified to strain level to ascertain their level of pathogenicity and public health risk they may pose.

Conclusion

The incidence of pathogenic microbes carrying multi-drug resistance genes in street vended RTE fruits and fruit salads is a growing public health concern that will continue to increase into the forseable future. We evaluated street vended RTE fruits and fruit salads for the presence of *E. coli* isolates and also evaluated the antimicrobial and drug potentials of the extracts and bioactive compounds of *T. tetraptera* using in-vitro and in-silico approaches, respectively. The abundance of the isolates varied according to fruit types and location. The isolates showed resistance to lower concentrations of the extracts and that of trimethoprim. The result of the GC-MS analysis revealed a total of twenty-eight compounds, out of which 13 passed Lipinski's ROF without any violation. ADMET analysis favourable pharmacokinetic properties, especially the absorption, metabolism and toxicity profiles by the bioactive compounds. Predicted target showed that the bioactive compounds could target the enzyme, dihydropteroate synthase. Docking scores returned favourable binding scores and interactions that were comparable to that trimethoprim. Put together, the findings indicates that the bioactive compounds from this edible pod *T. tetraptera* could be exploited further as potential lead compounds capable of interfering with the function of dihydropteroate synthase in *E. coli.*

Abbreviations

ADMET: Absorption, distribution, metabolism, excretion and toxicity.

BBB: Blood brain barrier

CNS: Central Nervous System

GC-MS: Gas chromatography-mass spectrophotometer

MW: Molecular weight

NIST: National Institute Standard and technique

PDB: Protein Data Bank

RCSC: Research Collaboratory for Structural Bioinformatics

ROF: Rule of Five

SMILES*: Simplified Molecular-Input Line-Entry System*

WHO: World Health Organization

^{o}C: Degree Celsius

g: Grams

h: Hour

min: Minutes

mm: Milliliters

mm: Millimeter

%: Percentage

mg/ml: Milligram/milliliters

2-D: 2-dimension

Declarations

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No funding was gotten for this study.

Availability of data and materials

All the data generated during the course of the study are contained in this article.

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Ethical approval and consent to participate

Given that the microbiological analyses of this study were performed at Arthur Jarvis University Biological Sciences Department, ethical approval was obtained for this study from the Research and Linkages Committee of Arthur Jarvis University (No: AJU/RC/9/22).

Consent for publication

Not applicable.

Competing interests

The authors do declare that there is no competing interest.

Authors' contributions

The study was conceptualized and design by GPB and UOE. All the microbiological analyses were handled by GPB, UOE, JCU, AAOE and AYO (isolation, identification and sensitivity testing). UOE, GPB and ENM handled SMILES strings generation, ADMET prediction, ligand and protein preparations and molecular docking jointly. All the results were jointly validated by all the authors. Initial manuscript draft was done by UOE, GPB and AYO while editing of manuscript draft was carried out by all the authors. All authors took turns to read the manuscript, and approved it for publication.

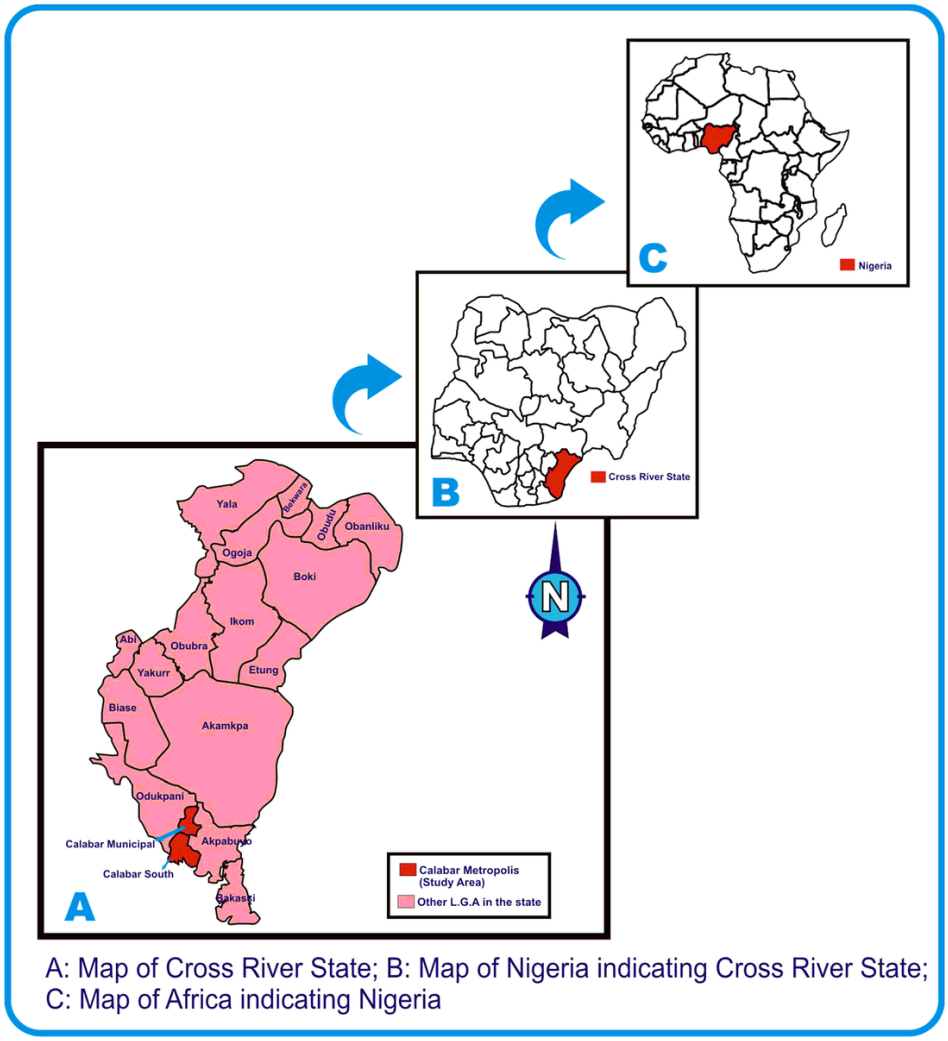
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Figures



A: Map of Cross River State; B: Map of Nigeria indicating Cross River State; C: Map of Africa indicating Nigeria

Figure 1

Maps show Cross River State, Nigeria and Africa [27].

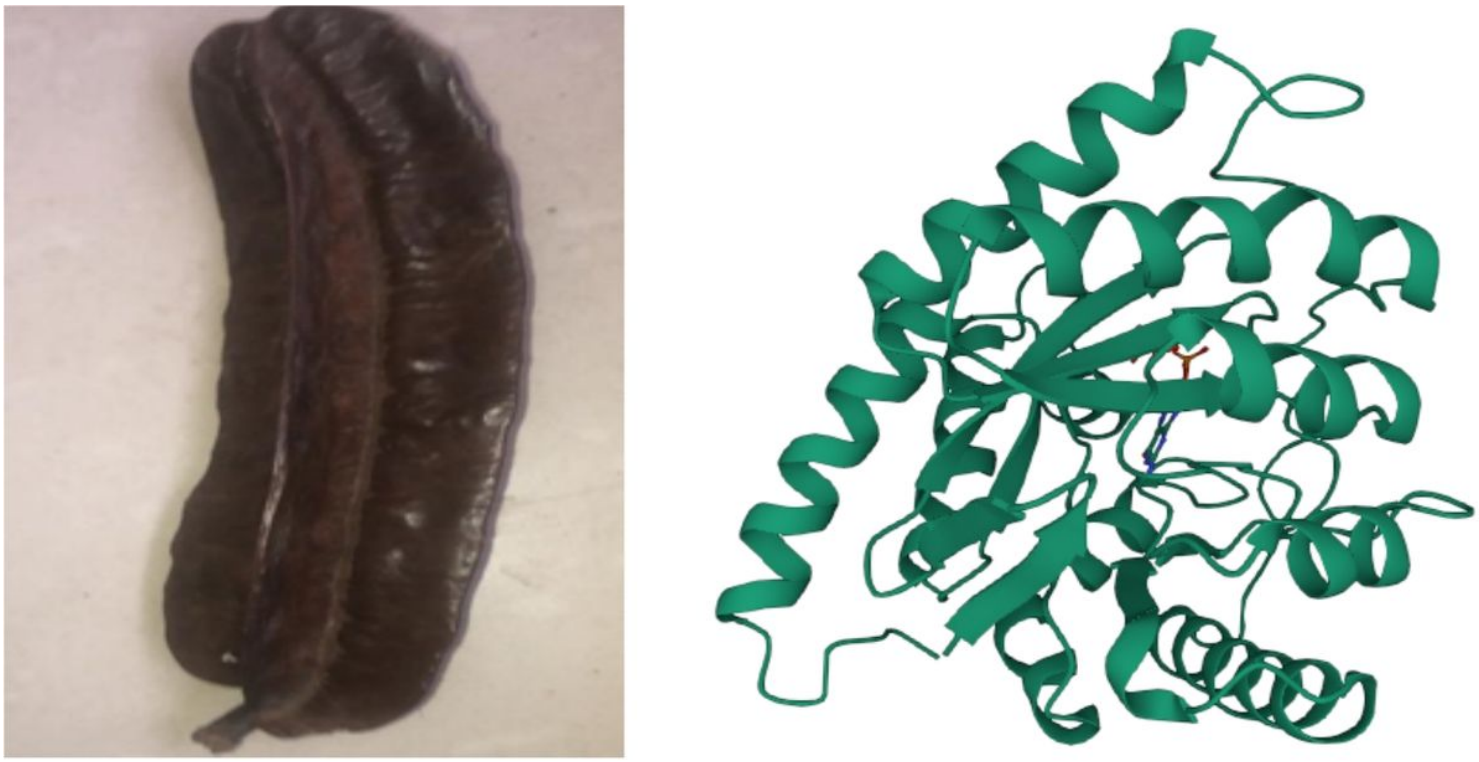


Figure 2

Whole pod (left) of the *T. tetraptera* sample used in the study and the 3D crystal structure of a binary complex of *E. coli*dihydropteroate synthase (right) retrieved from protein data bank.

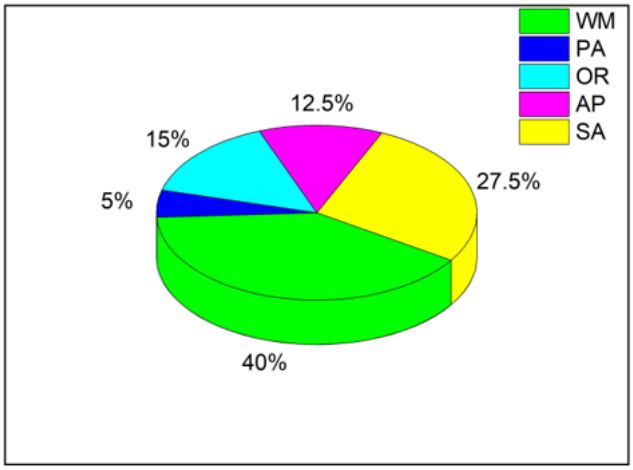


Figure 3

Distribution of the isolated *E. coli* according to fruit type.

Key: WM = Water melon, PA = Pineapple, OR = Orange, AP = Apple and SA = Salad (fruit).

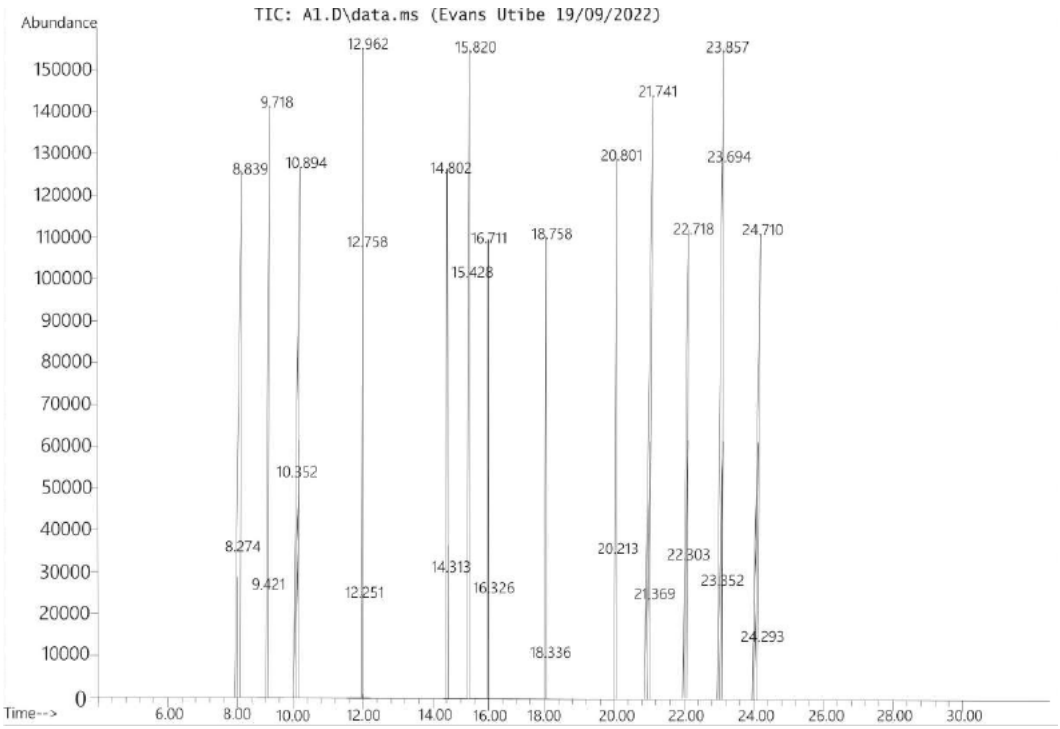


Figure 4

GC-MS chromatogram of the bioactive compounds from *T. tetraptera*



Figure 5

a: Target prediction for ligands I to VI. Key: Key: I = Methone, II = Lemonene, III = Octanol, IV = Menthol, V = Dihydrocarvone, VI = Piperitone.

b: Target prediction for ligands VII to XII and trimethoprim. Key: VII = Terpinene-4-ol, VIII = Eugenol, IX = 15-hydroxypentadecanoic acid, X = Carpaine, XI = T-cadinol, XII = Alpha-ocimene and XIII = Hexadecanoic acid and XIV = Trimethoprim

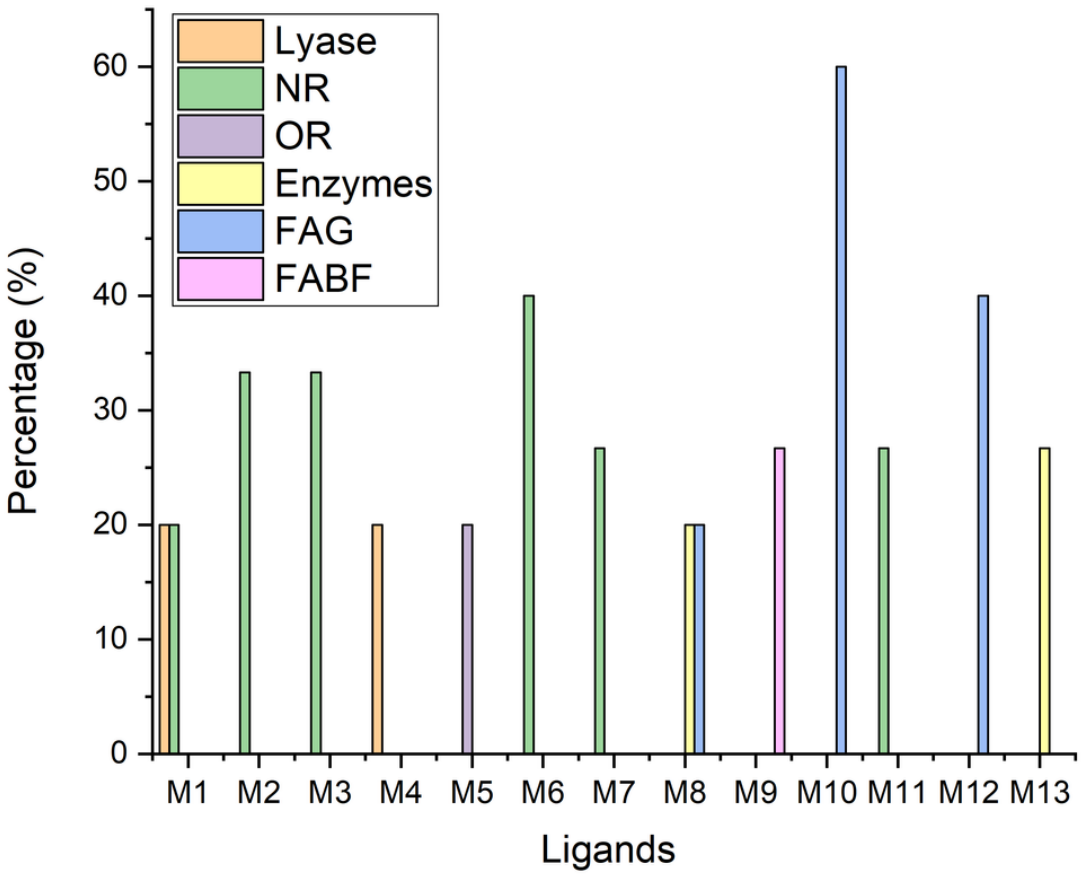


Figure 6

Top predicted targets for the various ligands.

Key: M1 to M13 = Methone, Lemonene, Octanol, Menthol, Dihydrocarvone, Piperitone, Terpinene-4-ol, Eugenol, 15-hydroxypentadecanoic acid, Carpaine, T-cadinol, Alpha-ocimene and Hexadecanoic acid, respectively. FAG = Family A G protein coupled receptor, FABF = fatty acid binding family, OX = Oxidoreductase and NR = Nuclear receptor.

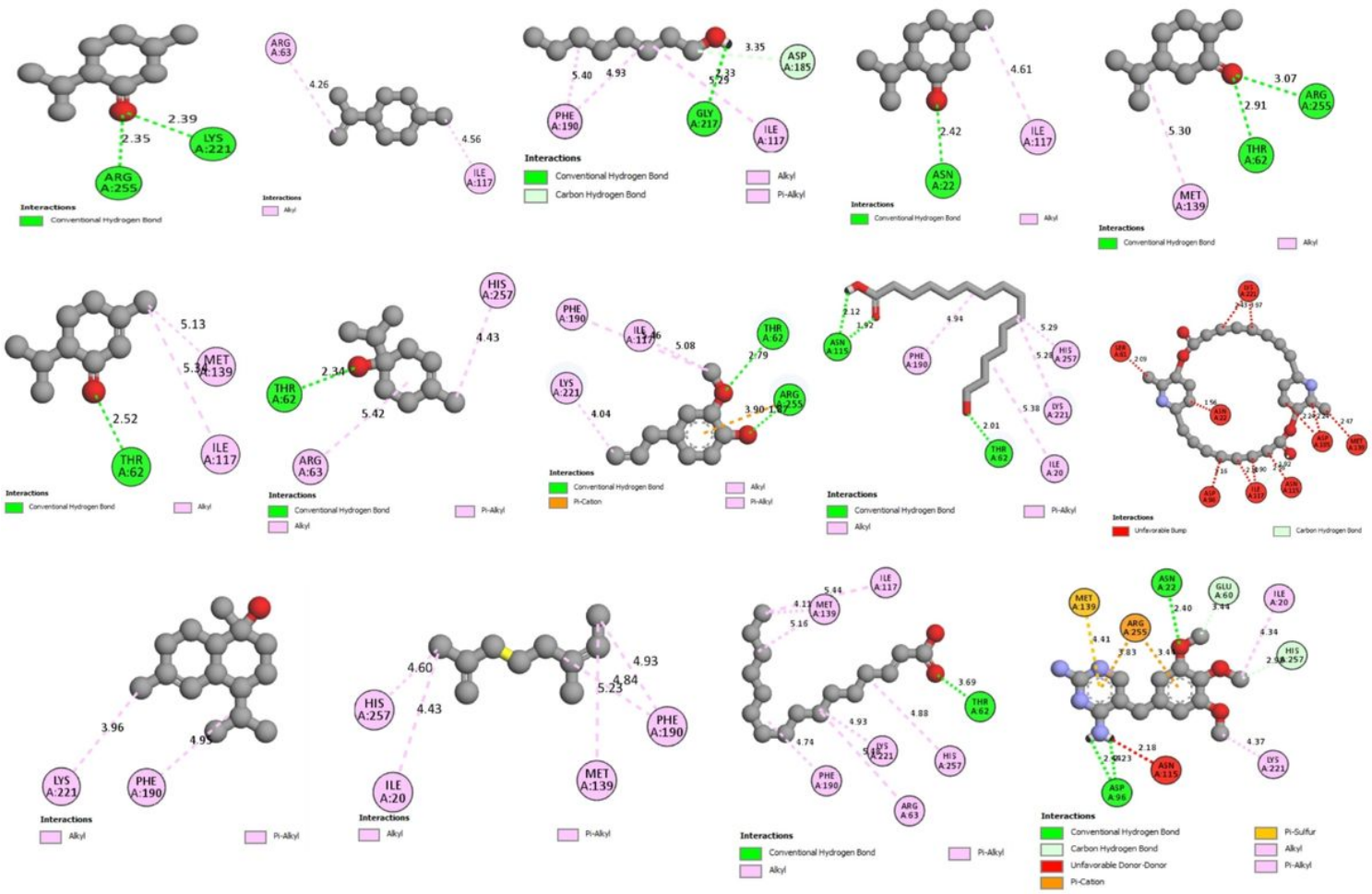


Figure 7

Docking outputs for the various ligands and trimethoprim visualized in 2-D using Biovia Discovery Studio 21 . Key: I = Methone, II = Lemonene, III = Octanol, IV = Menthol, V = Dihydrocarvone, VI = Piperitone, VII = terpinene-4-ol, VIII = eugenol, IX = 15-hydroxypentadecanoic acid, X = carpaine, XI = T-cadinol, XII = alpha-ocimene and XIII = hexadecanoic acid and XIV = Trimethoprim