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# Chemical, Antioxidant and Antibacterial Assessment of Clove *(Syzygium aromaticum)* Seed Extract and *in-silico* Pharmacokinetic Exploration of the Prominent Compounds

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**ABSTRACT**: The objective of this paper was to characterize, screen antioxidant, antibacterial and *in-silico* activities of petroleum ether extract of the seeds of *Syzygium aromaticum* plant using appropriate standard methods. Data obtained reveals the presence of ten compounds which belong to different classes of organic compounds comprising terpenes and terpenoids (50.512 %), methoxyphenol (17.232 %), glycoside (15.426 %), phenol ester (7.806 %), benzoic acid (5.423 %) and hydrocarbon (3.602 %). The extract did not record appreciable antioxidant activity in comparison with vitamin C used as a standard. The antibacterial activity screening against three gram-negative (*Escherichia coli*, *Klebsiella pneumonia*e and *Shigella flexneri*) and two gram-positive (*Streptococcus pneumoniae* and *Staphylococcus epidermidis*) bacteria organisms showed that the extract possessed marked antibacterial activity against the test organisms more than the gentamicin used as a standard antibacterial agent. The greatest activity was shown against *S. epidermidis* while the least activity was shown against *E. coli*. The presence of high level of terpenes and terpenoids, phenolic compounds and glycoside in the extract may be responsible for the high antibacterial activity demonstrated by the extract thereby giving credence to the use of *S. aromaticum* seed extract in the treatment of infections in herbal medicine. *In silico* ADME/pharmacokinetics activity was assessed using SWISSADME online server. The compounds showed good pharmacokinetic properties, such as high blood-brain barrier (BBB), high human gastrointestinal absorption (HIA), oral bioavailability and non-inhibition of cytochromes P450 (CYP). The findings of this study significantly increased the relevance of these compounds as promising first targets for the treatment of drug-resistant bacteria. This may help pharmacologists and other medicinal chemists create and synthesize even more potent drug candidates.

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Plants provide man with sources of materials used as food and drugs and many of these plants have been found to possess this dual usefulness (Igwe and Okwu, 2013a). One of such plants is *S. aromaticum* which is called Uda in Southeast Nigeria where it is mainly used as a spice and also as a drug. *S. aromaticum* belongs to the Myrtaceae family and has been reported to be used in traditional herbal medicine for the treatment of burns, wounds, tooth infections and toothache, vomiting, flatulence, nausea, liver, bowel and stomach disorders; and as a stimulant for the nerves (Batiha *et al.*, 2020). The plant has also been reported as a traditional remedy for scabies, cholera, malaria, and tuberculosis (Batiha *et al.*, 2020). Free radicals are highly reactive molecules with an unpaired electron and are produced by radiation or as by-products of metabolic process (Igwe and Okwu, 2013). Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure

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to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals (Lobo *et al.*, 2010). The search for organic antioxidants has become necessary since most of the synthetic antioxidants have been reported to be mutagenic (Igwe and Akabuike, 2016). Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitor, synergist, and metal-chelating agents (Lobo *et al.*, 2010). Several studies on the *in silico* characterization of secondary metabolites that may be important in microbial pathogenicity or may play a role in the regulation of metabolic processes have been conducted in the last few years (Otuokere *et al*., 2019, Otuokere *et al.*, 2020, Nwankwo *et al*., 2022; Otuokere *et al*., 2022a, Otuokere *et al.*, 2022b). The search for a more potent compound with increased bioactivities, or the development of new drugs, has been proven to be an expensive and time-consuming process (Abdullahi *et al*., 2020). The idea of ADME/pharmacokinetics, a branch of computational chemistry, may expedite the process of finding novel compounds while lowering synthesis costs (Abdullahi *et al.*, 2020). *In-silico* characteristics of a molecule, such as size, solubility, polarity, flexibility, lipophilicity, and saturation, provide crucial insights into whether the molecule may be used as a drug (Isyaku *et al.*, 2020).

*S. aromaticum* are used for many medicinal purposes and in the perfume industry. It is one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties (Cortés-Rojas, 2014; Batiha *et al.*, 2020). Several reports have documented the antibacterial, antiviral, anticarcinogenic, and antifungal activities of some aromatic herbs including *S. aromaticum* (Shan *et al.*, 2005). In view of the fact that most of the aforementioned traditional therapeutic claims are documented for the leaves and flowering buds, the objective of this paper was to characterize, screen antioxidant, antibacterial and *in-silico* activities of petroleum ether extract of the seeds of *Syzygium aromaticum* plant.

## MATERIALS AND METHODS

*Collection of Plant Materials:* The seeds of *S. aromaticum* were bought from Orie-ugba market in Umuahia North, Abia State, Nigeria, in the month of March, 2023. They were identified and authenticated at the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike.

*Sample Preparation:* The seed endosperms were obtained and washed thoroughly with water and allowed to dry. They were milled using an electric blender and was thereafter stored in an airtight container prior to extraction.

*Extraction of Phytochemicals:* The extraction process followed the procedure as reported by Igwe and Echeme (2013), with minor modifications. Soxhlet extraction method was used. 20 g of the pulverized sample was wrapped in a porous paper (Whatman No.1 filter paper). The wrapped sample was put in a soxhlet reflux flask containing 200 ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through an electro-thermal heater, it vaporized and condensed into the reflux flask. The wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over thus carrying the extracts from the sample down to the boiling flask. This process was allowed to go on repeatedly for about 4 h. The solvent was recovered using rotary evaporator and the extract was dried in the oven at 600 ℃ for 3 min to remove any residual solvent.

*Gas Chromatography/Mass Spectrometry Analysis:* An Agilent 6890N gas chromatography equipped with an auto-sampler connected to an Agilent mass spectrophotometric detector was used. The protocol employed here is as reported by Igwe and Abii (2014). 1 µl of sample extract was injected in the pulsed spitless mode onto a 30 m x 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 µm. Helium gas was used as a carrier gas and the column head pressure maintained at 20 psi to give a constant of 1 ml/min. Other operating conditions were preset. The column temperature initially held at 55$^{\circ}$C for 0.4 min, was increased to 200$^{\circ}$C at a rate of 25$^{\circ}$C /mins, then to 280$^{\circ}$C at a rate of 8$^{\circ}$C/mins and to final temperature of 300$^{\circ}$C at a rate of 25$^{\circ}$C/mins, held for 2 mins. All solvents used were 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).

*Antioxidant Activity Determination:* The free radical scavenging activity of the sample was determined using the 1,1-diphenyl-2-picrylhydrazyl ($\alpha$, $\alpha$-diphenyl-$\beta$-picrylhydrazyl; DPPH) method as reported by Igwe and Onuoha, (2016). 1.0 g of DPPH, a stable radical was dissolved in 100 ml of methanol. 3.0 ml of different concentrations of the test sample were added to 3.0 ml of a 0.004 % methanol solution

of DPPH and incubated for 30 minutes at room temperature. The decrease in absorbance of the solution brought about by the test samples was measured at 517 nm using a spectrophotometer. Ascorbic acid, which is a known antioxidant agent (Igwe, 2014) was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using the equation 1:

$$\%\, DPPH = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] \times 100 \quad (1)$$

Where; DPPH = inhibition of DPPH radical; $A_{blank}$ is the absorbance of the control reaction solution (containing all reagents except the test sample); $A_{sample}$ is the absorbance of the test sample

*Antibacterial Activity Screening Via Cell Concentration Assay:* The bacteria organisms used for the *in vitro* antibacterial screening were *Escherichia coli* (Gram-negative), *Klebsiella pneumonia* (Gram-negative), *Shigella flexneri* (Gram-negative), *Streptococcus pneumonia* (Gram-positive) and *Staphylococcus epidermidis* (Gram-positive). The test organisms were clinical isolates of human pathogens obtained from stock cultures at the Federal Medical Centre, Umuahia, Abia State, Nigeria. Tenfold serial dilution of the samples was prepared. Sterile water was used as diluent. A 9.0 mL amount of diluent was placed into each of 9 sterile test-tubes. The samples were mixed uniformly and with a sterile 1-mL micropipette, 1.0 mL was transferred into the first tube of diluent. This was done for the remaining dilutions in the same way, using a fresh pipette tip for each. Starting with the greatest dilution, 1.0 mL amounts of each dilution was pipetted into each of the three test tubes. 10 mL of clear nutrient broth and yeast extract broth melted and cooled to 45-50 ℃ was then poured aseptically into each of the test tubes and mixed for about ten seconds. The broth was allowed to set and incubated for 24 h at 37 ℃ in an incubator (Gallenkamp England). The viable cell concentration of bacterial isolates was evaluated after 24 h incubation through tracking the optical density of the cell colonies at 600 nm (OD600) using a UV spectrophotometer (K300, Jenway England), from the bacterial broth culture (Almeida *et. al*., 2013). Gentamicin was used as a standard antibacterial agent. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for the extract were determined.

*In silico Studies:* The PDBs of the most abundant compounds, 2-methoxy-3-(2-propenyl)-phenol and ethyl-α-d-glucopyranoside were downloaded from PubChem. They have been abbreviated as MPP and EGP, respectively. Lipinski's Rule of Five (RO5), pharmacokinetic analysis, and ADME were assessed using the web server of Swiss ADME (Daina *et al*., 2017).

## RESULTS AND DISCUSSION

The GC/MS chromatogram of the seed extract of *S. aromaticum* is shown in Figure 1. Only ten phytochemical compounds were identified in the seed extract as shown in Table 1. The antioxidant activity of the seed extract of the plant is shown in Table 2 while Table 3 shows its antibacterial activity.

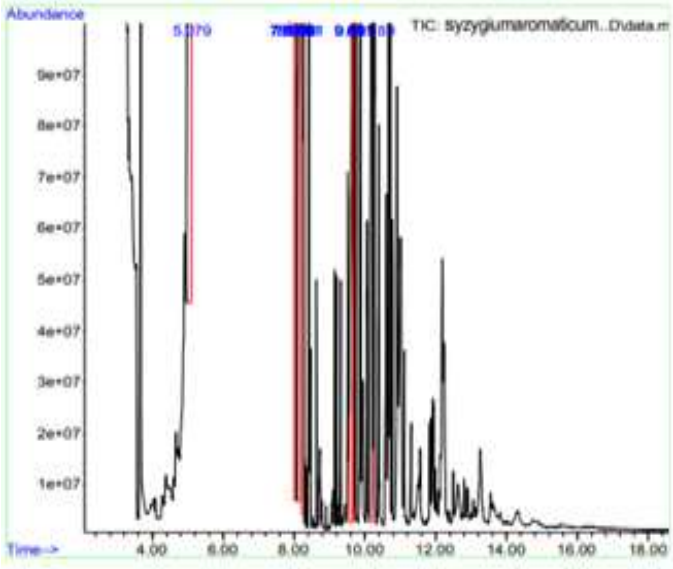


**Fig. 1:** GC/MS chromatogram of *S. aromaticum* seed extract

**Table 1:** Phytochemicals from the GC/MS analysis of *S. aromaticum* seed extract

| Peak No. | Phytochemical Components | Retention Time (min.) | Class of Compound | Percentage Composition (%) |
|---|---|---|---|---|
| 1 | 2-Methoxy-3-(2-propenyl)-phenol | 5.079 | Methoxyphenol | 17.232 |
| 2 | Eugenol | 7.832 | Monoterpenoid | 7.666 |
| 3 | β-Caryophyllene | 7.872 | Sesquiterpene | 8.894 |
| 4 | α-Humulene | 7.958 | Sesquiterpene | 10.853 |
| 5 | Ethyl-α-d-glucopyranoside | 8.038 | Glycoside | 15.426 |
| 6 | Clionasterol acetate | 8.181 | Triterpenoid (phytosterol) | 14.835 |
| 7 | α-Farnesene | 8.261 | Sesquiterpene | 8.264 |
| 8 | 1-Methylcycloundecene | 9.651 | Hydrocarbon | 3.602 |
| 9 | Phthalic acid | 9.691 | Benzoic acid | 5.423 |
| 10 | Eugenyl acetate | 10.189 | Phenol ester | 7.806 |

**Table 2:** Antioxidant activity of *S. aromaticum* seed extract

| Concentration (µg/ml) | Antioxidant activity (%) | |
|---|---|---|
| | Seed extract | Vitamin C |
| 25 | - | 80.90 ± 4.343 |
| 50 | 1.05 ± 1.97 | 95.16 ± 1.549 |
| 100 | 2.37 ± 0.78 | 97.63 ± 0.068 |
| 200 | 4.52 ± 0.80 | 97.81 ± 0.104 |
| 400 | 8.49 ± 0.61 | 98.28 ± 0.306 |

*Data are mean ± standard deviation of triplicate determinations*

**Table 3:** Antibacterial activity of *S. aromaticum* seed extract

| Test Microorganism | *S. aromaticum* seed extract | Gentamicin | Water | MIC | MBC |
|---|---|---|---|---|---|
| *E. coli* | 0.34±0.07 | 0.13±0.04 | 0.00 | 0.250 | 0.500 |
| *K. pneumoniae* | 0.57±0.30 | 0.05±0.03 | 0.00 | 0.250 | 0.500 |
| *S. flexneri* | 0.60±0.25 | 0.04±0.02 | 0.00 | 0.125 | 0.250 |
| *S. pneumoniae* | 0.49±0.14 | 0.03±0.00 | 0.00 | 0.125 | 0.250 |
| *S. epidermidis* | 0.74±0.04 | 0.05±0.02 | 0.00 | 0.125 | 0.250 |

*Data are mean ± standard deviation of triplicate determinations*

Table 4 represents the drug-likeness prediction of test compounds present in *S. aromaticum* seeds. Some ADME parameters for the prediction of prominent compounds are present in Table 5**.** The interaction of test compounds with cytochrome P450 (CYP) is depicted in Table 6. The bioavailability radar of MPP and EGP are presented in Figure 2 while Figure 3 represents the BOILED-Egg diagram of MPP and EGP.

**Table 4:** Drug-likeness prediction of phytochemicals from *S. aromaticum* seed

| Compounds | Mol. weight (g/mol.) | HB Acceptor | HB Donor | Lipophilicity LogP | Molar refractivity | No. of violations |
|---|---|---|---|---|---|---|
| MPP | 164.20 | 2 | 1 | 2.01 | 49.06 | 0 |
| EGP | 208.21 | 6 | 4 | -2.07 | 45.27 | 0 |

**Table 5**: Some ADME Parameters prediction of phytochemicals from *S. aromaticum* seed

| Compound | TPSA (Å²) | Water Solubility Log *S* (ESOL) | Bio. Avail. Score | Med. Chem. (PAIN) alert | Synthetic Accessibility |
|---|---|---|---|---|---|
| MPP | 29.46 | -2.67 | 0.55 | 0 | 1.60 |
| EGP | 99.38 | 0.50 | 0.55 | 0 | 4.41 |

**Table 6:** Interaction of *S. aromaticum* seed phytochemicals with cytochromes P450 (CYP)

| Compounds | CYP1A2 | CYP2C19 | CYP2C9 | CYP2D6 | CYP3A4 | Log $K_p$ (skin permeation) (cm/s) |
|---|---|---|---|---|---|---|
| | | | Inhibitor | | | |
| MPP | Yes | No | No | No | No | -5.45 |
| EGP | No | No | No | No | No | -9.19 |

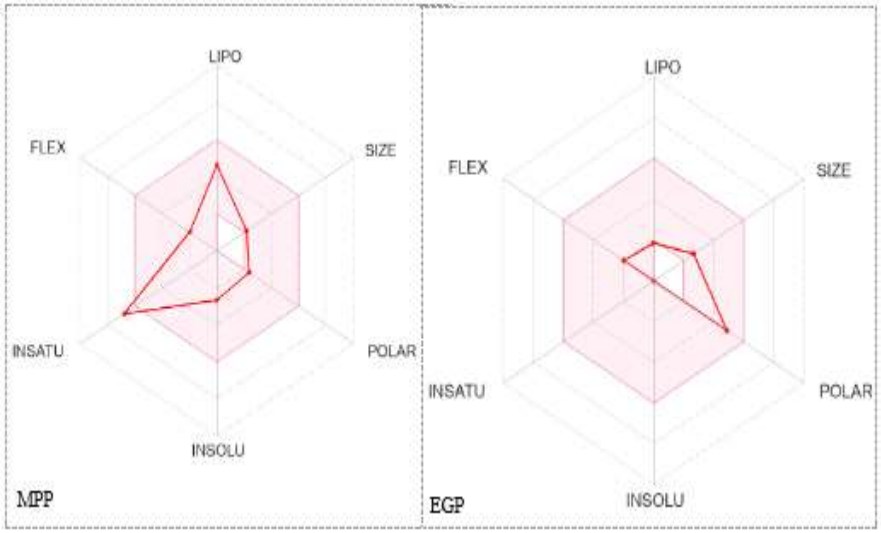


**Fig 2:** The bioavailability radar of MPP and EGP

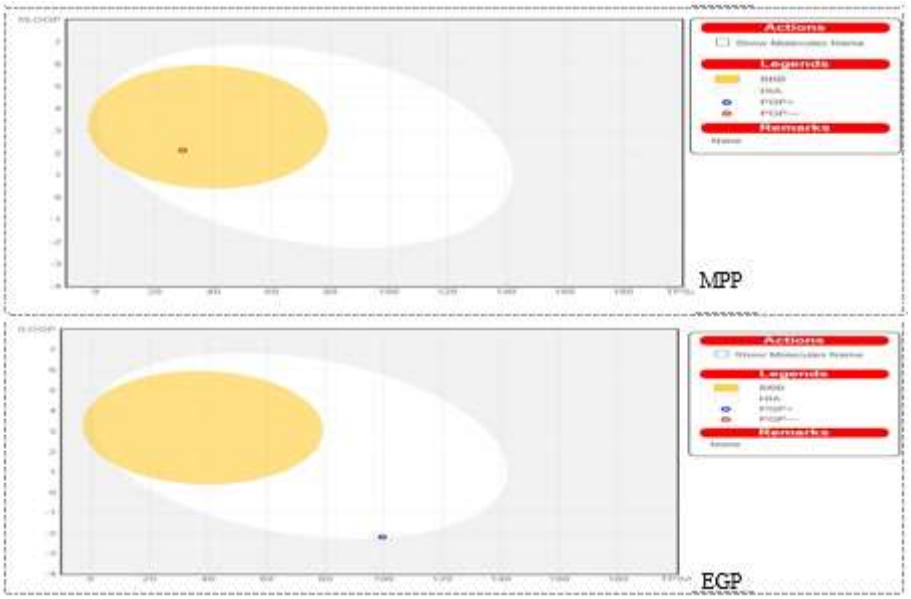


**Fig 3**: BOILED-Egg diagram of MPP and EGP

The GC/MS chromatogram of *S. aromaticum* seed extract (Figure 1) shows the presence of ten phytochemical compounds as identified and shown in Table 1. The compounds comprised terpenes and terpenoids (50.512 %), methoxyphenol (17.232 %), glycoside (15.426 %), phenol ester (7.806 %), benzoic acid (5.423 %) and hydrocarbon (3.602 %). The extract chiefly contained terpenes and terpenoids. The compounds found in the seed extract in decreasing order of composition were 2-methoxy-3-(2-propenyl)-phenol, ethyl-α-d-glucopyranoside, clionasterol acetate, α-humulene, β-caryophyllene, α-farnesene, eugenyl acetate, eugenol, phthalic acid and 1-methylcycloundecene. It has been reported that terpenes and terpenoids possess a wide range of biological activities including anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic activities (Masyita *et al*., 2022). For instance, eugenol has been reported to possess pain-relieving, antioxidant, anti-tumor, anti-depressant, antispasmodic, anti-inflammatory, and anti-microbial properties (Nejad *et al*., 2017). The extract did not record appreciable antioxidant activity in comparison with vitamin C used as a standard (Table 2). The antibacterial activity screening against three gram-negative (*E. coli*, *K. pneumonia*e and *S. flexneri*) and two gram-positive (*S. pneumoniae* and *S. epidermidis*) bacteria organisms showed that the extract possessed marked antibacterial activity against the test organisms more than the gentamicin used as a standard antibacterial agent (Table 3). The greatest activity was

shown against *S. epidermidis* while the least activity was shown against *E. coli*. The presence of high level of terpenes and terpenoids, phenolic compounds and glycoside in the extract may be responsible for the high antibacterial activity demonstrated by the extract thereby giving credence to the use of *S. aromaticum* seed extract in the treatment of infections in herbal medicine. The RO5, proposed by Lipinski, aids in assessing if a substance with a specific bioactivity has the necessary chemical and physical characteristics to be used as an oral medication. For every molecule in the drug-likeness forecast, there was no violation (Table 4). The test compounds meet RO5 requirements. Minimal compound attrition is anticipated for the test compounds for future drug development research (Otuokere *et al.,* 2022a; Otuokere *et al.,* 2022b; Nwankwo *et al.,* 2022; Asuquo *et al.,* 2023; Amaku *et al.,* 2020).

For the Lipinski rule (Lipinski *et al.,* 2012), a good drug should have a topological polar surface area (TPSA) of less than 140 $Å^2$. This is one of the most significant chemical descriptors that correlate strongly with pharmacokinetic parameters. The test compounds' TPSA was less than 140 Å2 (Table 5).
It is much easier to handle and formulate drugs when the molecule is soluble (Ritchie *et al.,* 2013). This is one of the main benefits of solvent-based drug development. According to Ottaviani*et al.* (2010), solubility is a significant factor affecting absorption in research efforts that aim for oral delivery. According to Savjani*et al.* (2012), a medication intended for parenteral administration must also have high water solubility in order to provide an adequate amount of the active ingredient in the tiny amount of the prescribed pill. An ESOL model water solubility profile (Delaney, 2004) for the test compounds is shown in Table 5. The solubility profile indicated MPP was soluble in aqueous whereas EGP was insoluble.

The bioavailability score (Martin, 2005) calculates the probability that a chemical will have at least 10% oral bioavailability in rats using the predominant charge of a rat model at biological pH. This predicts the probability that a material will have F > 10%. Martin (2005) stated that a molecule is generally considered a potential therapeutic candidate if its bioavailability score is at least 0.10. The oral bioavailability of MPP and EGP was predicted to be 0.55 and 0.55, respectively (Table 5). PAINS (pan assay interference compounds), which are also known as frequent hits or promiscuous compounds, are molecules with substructures that show strong reactions in assays no matter what protein target they are tested against. Our results (Table 5) showed that there are no promiscuous compounds that can cause drug interference. The score is normalised between 1 (easy synthesis) and 10 (extremely difficult synthesis) for synthetic accessibility. The test compounds MPP and EGP are anticipated to be easily synthesised based on the synthetic accessibility test score (Table 5). Understanding the interactions between chemicals and cytochromes P450 (CYP) is also crucial. This isoenzyme superfamily plays a crucial role in drug clearance via metabolic biotransformation. Research suggests that P-gp and CYP can work together to metabolise small compounds in a way that enhances tissue and organism protection (Van Waterschoot and Schinkel, 2011). One main reason for pharmacokinetics-related drug-drug interactions (Hollenberg, 2002) is inhibition of these isoenzymes. This can cause toxic or other unwanted side effects because the drug or its metabolites don't get cleared out as quickly or as much (Kirchmair*et al.,* 2015). It has been determined that there are several CYP isoform inhibitors. Some substances selectively target particular isoenzymes, while others impact distinct CYP isoforms (Veith*et al.,* 2009). Predicting which isoforms will be impacted and the likelihood that the compounds will inhibit CYPs to create meaningful drug interactions are therefore crucial steps in the drug discovery process. Our research indicated that MPP did not inhibit CYP2C19, CYP2C9, CYP2D6 and CYP3A4. EGP did not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 (Table 6). Potts and Guy (1992) reported that the more negative the log Kp (with Kp in cm/s), the less skin permeant the molecule is. Log Kp values for MPP and EGP were negative. This suggested that they were not skin-permeable.

Figure 2 displays the bioavailability radar for a quick assessment of drug-likeness. The following six physicochemical characteristics were considered: size, polarity, solubility, flexibility, saturation, and lipophilicity. Descriptors taken from Ritchie *et al*. (2011) and Loverin*get al.* (2009) were used to create a physicochemical range on each axis. A pink area delineates the range within which a molecule's radar plot must fall completely in order to qualify as drug-like. In our research work, EGP was orally bioavailable, while MPP was not orally bioavailable because it was slightly saturated.

By using the BOILED-Egg (Figure 3), it is easy to get a sense of how the positions of the molecules in the WLOGP-versus-TPSA referential affect the blood-brain barrier (BBB) and human gastrointestinal absorption (HIA). The possibility of brain penetration is higher in the yellow zone (yolk) than in the white area, which has a high chance of passive absorption by the gastrointestinal tract (Ritchie *et al*., 2011; Loverin*get al*., 2009). White areas and yolk areas are

not mutually exclusive. Also, the spots are red if they are thought to not bind to P-gp (PGP−) and blue if they are thought to be actively pushed out by the permeability glycoprotein P-gp (PGP+). MPP had significant BBB absorption in this study and were predicted to be non-substrates of P-gp (PGP−) whereas EGP showed HIA absorption and actively effluxed from glycoprotein P-gp (PGP+).

*Conclusion:* It has been concluded that the prominent compounds found in *S. aromaticum* seeds have antibacterial properties. The presence of high level of terpenes and terpenoids, phenolic compounds and glycoside in the extract may be responsible for the high antibacterial activity. All of the compounds have shown positive properties for Lipinski's requirements, such as good solubility in the aqueous medium (Log S) and TPSA<140, as well as good permeability in biological membranes and the blood-brain barrier. The *in silico* prediction's accuracy and the experimentally demonstrated antibacterial properties agreed well. The pharmacokinetic properties predictions revealed that all the designed inhibitors possessed excellent pharmacological properties. On these accounts, the hypothetical designed inhibitors could be employed for further experimental investigations, *in vivo* evaluations and other pharmacological assessments before they could be optimized as novel antibacterial agents/drug candidates.

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