



ORIGINAL ARTICLE

In silico, *in vitro* antioxidant and density functional theory based structure activity relationship studies of plant polyphenolics as prominent natural antioxidants

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Abstract In the present study, different extracts of stem bark of *Shorea tumboagaia* and roots of *Syzygium alternifolium* were screened for total phenolic content (TPC), total flavonoid content (TFC) and *in vitro* DPPH radical scavenging activity to isolate the natural antioxidants. The bioassay-guided fraction of stem bark of *Shorea tumboagaia* yielded three compounds, piceatannol (ST-1), resveratrol-12-C-β-D-glucopyranoside (ST-2) and hopeaphenol (ST-3). Similarly, a systematic phytochemical examination of extracts of *Syzygium alternifolium* roots had resulted five compounds, 5,7,8,5'-tetramethoxy-3',4'-methylenedioxyflavone (SA-1), 5-hydroxy-4',7-dimethoxy-6,8-di-C-methylflavone (SA-2), quercetin 7-methylether (SA-3), kaempferol 7,4'-dimethylether 3-O-β-D-glucopyranoside (SA-4) and taxifolin 3-O-α-L-rhamnopyranoside (SA-5). Structures of the isolates were established using UV, IR, Mass, ¹H and ¹³C NMR spectral studies. Density Functional Theory (DFT) calculations, Molecular docking and Lipinski rule of five were conducted to explore the antioxidant activity of isolated compounds. SA-3 (37.52 μg/mL) and ST-1 (42.43 μg/mL) showed highest IC₅₀ values compared to the Ascorbic acid (45.97 μg/mL) and have

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highest electron affinities (EA eV) along with smallest HOMO-LUMO energy gap. Molecular docking and binding affinity studies with NADPH and SPSB2 proteins, revealed the prominent antioxidant activity of SA-3 and ST-1 with molecular interaction besides promising solvation energies. Hence, the two compounds may be useful for the treatment of oxidative damage related diseases.

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1. Introduction

Imbalance cell redox reactions are the origin for the formation of free radicals or reactive oxygen species (ROS) such as superoxide ($O^{\bullet-}$), peroxide ($^{\bullet}OOH$), singlet oxygens (O_2), hydroxyl radical ($^{\bullet}OH$) and nitric oxide (NO^{\bullet}) in human tissue cells during the metabolic process. In addition, food habits, life style process and environmental factors are may be other reasons for over production of ROS (Snezhkina et al., 2019; Zhou et al., 2016). These ROS in high concentration may capable to oxidize biomolecules such as proteins, fats, lipids, carbohydrates and nucleic acids which are present in human organism (Pelaez-Jaramillo et al., 2019; Young and Woodside, 2001). Conversely, lead to endogenous oxidative stress related disease such as hypertension, cancers, digestive ulcers, rheumatoid arthritis, non-inflammatory cancers, cardiovascular risks, aging and neurological disorders (Hertog, 1993; Nita and Grzybowski, 2016; Reaven and Witztum, 1996). Antioxidants are the substances which sustaining the balanced metabolic process by inhibiting detoxification chains of ROS (Anbudhasan et al., 2014). Consumption of dietary foods like vegetables, nuts, fruits, beverages, tea, coffee beans, leafy vegetables and traditional medicinal herbs which are rich in natural antioxidants can prevent such kinds of oxidative damages related diseases (Ariza et al., 2018; Battino et al., 2019; Pietta, 2000). Several synthetic drugs are also available in the pharmaceutical market to treat these oxidative damage related diseases, but they may result several adverse effects on human health due to their inherent toxicity (Saito et al., 2003). Hence, novel antioxidants are needed to identify from the natural source owing to prominent role in human life.

In recent years, the discovery and development of natural antioxidants from traditional plant sources is a great deal of attention due to their prominent role in the control or prevention of oxidation process and associated diseases (Battino et al., 2019; Rajaram et al., 2019). Plants are rich source of polyphenolics such as flavonoids, anthocyanins, pro-anthocyanins, carotenoids, catechins, caffeates, cinnamic acid derivatives, chalcones, stilbenes, tocopherols and certain types of nitrogenous compounds which enhance the quality of life through antioxidant principles (Li et al., 2014; Velioglu et al., 1998). Flavonoids are major plant constituents and few are reported as versatile natural antioxidants such as chalconaringenin, naringenin, kaempferol and quercetin derivatives, xanthohumol, isoxanthohumol, ginkgo flavone glycoside, silymarin, catechin and anthocyanin derivatives (Cao et al., 1997; Kashima, 1999). In view of the importance of medicinal plants and their phytoconstituents, the present work intended to the isolation of potent antioxidants from rare, endemic and endangered medicinal plants namely *Shorea tumbergaia* (Dipterocarpaceae) and *Syzygium alternifolium* (Myrtaceae). These plants were widely used in the traditional medicine and showed interesting biological properties, for example, ulcers, rheumatic pain (Babu et al.,

2016), antihyperglycemic activity (Kasetti et al., 2012), dysentery, diarrhea and cholera (Chitravadivu et al., 2009).

2. Materials and methods

2.1. Chemicals

Chemicals and organic solvents with analytical grade were purchased from Merck and used without further purification. Thin Layer Chromatography (TLC) was performed by using precoated silica gel 60 F₂₅₄ plates. Column chromatography was executed on silica gel (Acme) finer than 200 mesh (0.08 mm thickness). Melting points were determined using a Kofler hot stage apparatus and are uncorrected. EI mass spectra were obtained on Nermag R10-10 mass spectrometer, ESI-TOFMS and ESI-MS/MS spectra were recorded in positive mode on an API Q-STAR PULSA i of Applied Biosystems. UV spectra were measured in MeOH on a Shimadzu UV-1800 spectrophotometer. IR spectra on a Bruker AlphaEco ATR-FTIR spectrophotometer, and ¹H and ¹³C NMR spectra on Bruker Avance spectrometer operating at 400, 300 for ¹H and 100, 75 MHz for ¹³C, respectively using CDCl₃, DMSO *d*₆ and Me₂CO-*d*₆ with TMS as an internal standard. The UV absorbance was determined by using a Techcomp VIS-7200A visible spectrophotometer.

2.2. Plant materials

The stem bark of *Shorea tumbergaia* and roots of *Syzygium alternifolium* were collected from Seshachalam hill ranges of Andhra Pradesh, India and identified by Dr. K. Madhava Chetty, Assistant Professor and plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India and where voucher specimens (DG-111B & 117) are deposited.

2.3. Preparation of crude extracts for preliminary antioxidant activity studies

50.0 g of shade dried stem bark of *Shorea tumbergaia* and roots of *Syzygium alternifolium* powder material were taken in the thimble and are introduced into the Soxhlet apparatus and then extracted with 500 mL of hexane, acetone and methanol, successively. On evaporation of the solvent yielded the crude extracts. The crude extracts of hexane, acetone and methanol were screened for a) Total Phenolic Content (TPC) b) Total Flavonoid Content (TFC) and c) DPPH radical scavenging activity for their *in vitro* antioxidant activity.

2.4. Estimation of total phenolic content (TPC)

TPC of the plant extracts was determined using spectrophotometric method (Singleton et al., 1999). To the 1 mL of each

plant extract (1 mg/mL), 1 mL of Folin-Ciocalteu's reagent was added followed by addition of 10 mL of 7% Na₂CO₃ and 13 mL of sterilized double distilled water. The total mixture was incubated at 25 °C in the dark for about 90 mins. Subsequently, the absorbance was measured at 760 nm and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample. The absorbance of all the samples was evaluated in three replications.

2.5. Estimation of Total Flavonoid Content (TFC)

TFC of the plant extracts was resolved with colorimetric method using quercetin as standard (Kim et al., 2003; Rammohan et al., 2015). In this method, 1 mL of each plant extract (1 mg/mL) and 4 mL of distilled water placed in a 10 mL volumetric flask. At zero time, 0.3 mL of 5% NaNO₂ and 0.3 mL of 10% AlCl₃·6H₂O was added. After 5 min, 2 mL of 1 M NaOH was added to the mixture and then make the total volume up to 10 mL with sterilized double distilled water. The absorbance of well mixed sample solutions was measured at 510 nm and the results were stated as milligrams of quercetin equivalents (QE) per gram of dried sample. The absorbance of the experimental samples was measured in three replications.

2.6. DPPH radical scavenging assay

Free radical scavenging activity of plant extracts and the isolated compounds were carried out by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay (Morita et al., 2017). 1 mL of test sample with various concentrations (20, 40, 60, 80 & 100 µg/mL) was added to 2.0 mL of 0.1 mM DPPH dissolved in methanol. The mixture was vortexed for 1 min and then incubated for 30 min at room temperature. After incubation time, the absorbance was measured at 517 nm with ascorbic acid as the standard. The radical scavenging activity (RSA) was calculated using the equation

$$\%RSA = [(A_c - A_s)/A_c] \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.7. Extraction and isolation of chemical constituents

The shade dried and powdered stem bark (1.8 kg) of *S. tumbagaia* was exhaustively extracted with *n*-hexane, acetone and methanol at room temperature. The hexane extract has not been taken up for further examination as it didn't show any significant TPC content. The acetone extract (60 g) and the methanol extract (90 g), which have significant phenolic content (TPC) were found to be similar on TLC and hence combined and evaporated *in vacuo* to give a brownish yellow residue (150 g). It was chromatographed on a silica gel column eluting with *n*-hexane-ethyl acetate step gradient mixtures. The *n*-hexane-ethyl acetate, 4:6, 3:7 and 1:9 eluates on concentration afforded three brown solids, were designated as ST-1, ST-2 and ST-3, respectively.

The roots (2.1 kg) of *S. alternifolium* were shade dried, powdered and exhaustively extracted with *n*-hexane, acetone and methanol. The hexane extract on concentration under reduced pressure yielded a pale-yellow syrupy residue (10 g) and was

purified over a silica gel column using *n*-hexane-ethyl acetate step gradient mixtures as eluents. The hexane-ethyl acetate (7:3) eluent on concentration yielded a white solid (20 mg) which on crystallization from chloroform gave colourless needles (15 mg) designated as SA-1. The acetone extract was concentrated under reduced pressure to yield a dark green coloured residue (35 g) and it was purified over a silica gel column using *n*-hexane-ethyl acetate step gradient mixtures. The *n*-hexane-ethyl acetate eluates 8:2 and 6:4, on concentration afforded two yellow solids which on crystallization from methanol gave pale yellow needles (17 mg) and crystals (18 mg). It was designated as SA-2 and SA-3, respectively. The methanol extract was concentrated under reduced pressure to yield a greenish yellow viscous residue (57 g). It was purified over a silica gel column using *n*-hexane-ethyl acetate step gradient mixtures. The *n*-hexane-ethyl acetate eluates 7:3 and 1:1, on evaporation gave two yellow solid which on crystallization from methanol resulted in yellow crystals (17 mg) and colourless needles (15 mg). It was designated as SA-4 and SA-5, respectively.

2.8. Density Functional Theory (DFT) studies

In order to explain the antioxidant activity of all the isolated compounds, the electronic parameters such as Molecular Electrostatic Potential (MEP), HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital), their energy gap (ΔE), Ionization Potential (IP) and Electron Affinity (EA) were calculated by using DFT method. The theoretical calculations were performed using the GAMESS-US (Noro et al., 2012; Reddy et al., 2016) line up package with B3LYP (Becke's three-parameter exchange potential and the Lee-Yang-Parr correlation functional) with standard basis set 6-311G (d,p) and the lowest energy geometry were obtained using the semi-empirical method PM3. The electronic structures along with spin density distribution of HOMO and LUMO were presented in color-coded surface which gives the location of positive and negative electrostatic potentials. The electronic properties were computed and validated using Koopman's theorem (1934). According to this theorem, $IA = -E_{HOMO}$, ionization potential is negative to HOMO energy orbital which means to remove an electron from highest occupied orbital. In other way, the capability of HOMO orbital to reduce the free radicals by donating electron. Similarly, electron affinity is computed as, $EA = -E_{LUMO}$ i.e. which means the ability of LUMO orbital to capture a free radical. The energy gap ΔE is the difference between HOMO-LUMO orbitals of the isolated compounds, which specify the capacity of molecule involved in the free radical optimization mechanism (Borges et al., 2015). If the ΔE is small the intramolecular charge transfer is easy, and the molecule participate effectively in radical scavenging reaction (Islam, 2015).

2.9. Protein preparation

Crystal structures of NADH (PDBID: 1k4u) (You et al., 2017) and SPSB2 (PDBID: 3emw) (Kami et al., 2002) were retrieved from Protein Data Bank (PDB). Co-crystal ligands, water and ions were removed and hydrogens were added, energy minimization was employed using steepest descent algorithms (Guex and Peitsh, 1997).

2.10. Ligand preparation

Isolated flavonoids were drawn and 3D optimized, hydrogens were added and energy minimized using MarvinSkech. The refined and energy minimized molecules were used for docking studies. Lipinski rule was computed using Molsoft molecular property prediction.

2.11. Molecular docking

AutoDock Vina 4.05 was used to dock into the active site of target proteins (Trott and Olson, 2010; Wolf, 2009). Ligand molecules were uploaded and energy minimized with universal force field using conjugate-gradient algorithm with 200 run iterations and converted as PDBQT files. Docking grid box prepared and positioned to cover the binding pockets according to the location of key residues. The grid box and size were followed: for SH3 domain was center (X) = -1.46251269576, center (Y) = 5.26906821151, center (Z) = -1.16382932524, size (X) = 26.1067726284, size (Y) = 19.4079249122, size (Z) = 26.5021901293, for SPSB2 was center (X) = 11.48738 57579, center (Y) = 28.5127526796, center (Z) = 55.862505 5939, size (X) = 23.7570462719, size (Y) = 24.9365557258, size (Z) = 20.4731834919 and exhaustiveness was set at 8. Lamarckian genetic algorithm was used and docking parameters were set as follows: the number of individuals in the population was 150, maximum number of energy evaluations was 25,000, maximum number of generations was 27,000, top individual to survive to next generation was 1, gene mutation rate was 0.02, crossover rate was 0.8, Cauchy beta was 1.0 and genetic algorithm window size was set to 10.0. The best docked ligand conformations were saved, and the bond angles, bond lengths, and hydrogen bonding.

2.12. Born interaction energies and binding affinities

Generalized Born/Volume Integral (GB/VI) implicit solvent methods implemented in MOE determined binding affinities of lead molecules (Bhaskar et al, 2016). Van Der Waals, Coulomb electrostatic interactions and implement solvent interactions are termed as Born interaction energy which determined non-bonded interactions between the ligands and the protein. During the binding affinity assessment, ignored draining

energies, solvent molecules of the ligand and the protein. London scoring method was represented in kcal/mol unit for the binding affinity calculations. The binding pocket residues and the ligands were kept as flexible where binding pocket was kept as rigid to tether restraints to discourage gross movements. Energy minimization for the protein-ligand complex was employed and calculated binding affinity.

3. Statistical analysis

All the experiments were performed in triplicate (n = 3). The Statistical data were analyzed by using ANOVA software and expressed as mean ± SD. The values p < 0.05 were considered as significant.

4. Results

4.1. TPC and TFC studies

The phenolic content may contribute directly to the antioxidant activity. The total phenolic content in *n*-hexane, acetone and methanol extracts of *Shorea tumbergaia* and *Syzygium alternifolium* were determined spectrophotometrically according to the Folin–Ciocalteu method and calculated as gallic acid equivalents (GAE). Total phenolic content of acetone (STA) and methanol (STM) extracts of *S. tumbergaia* are 62.56 ± 0.9 and 64.67 ± 0.8 µg/mL of gallic acid equivalents, respectively. The result shows that the phenolic content present in both acetone and methanol extracts are significant and almost similar.

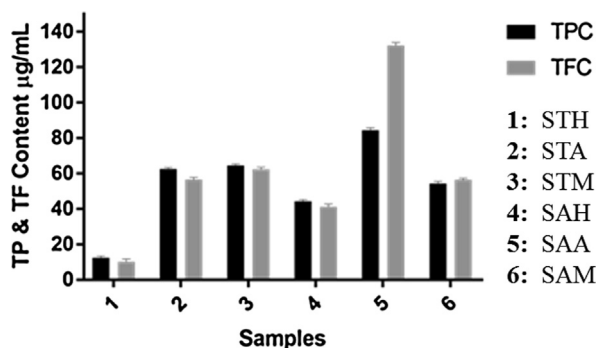


Fig. 1 Total phenol content and total flavonoid content in different extracts of *Shorea tumbergaia* (ST) and *Syzygium alternifolium* (SA).

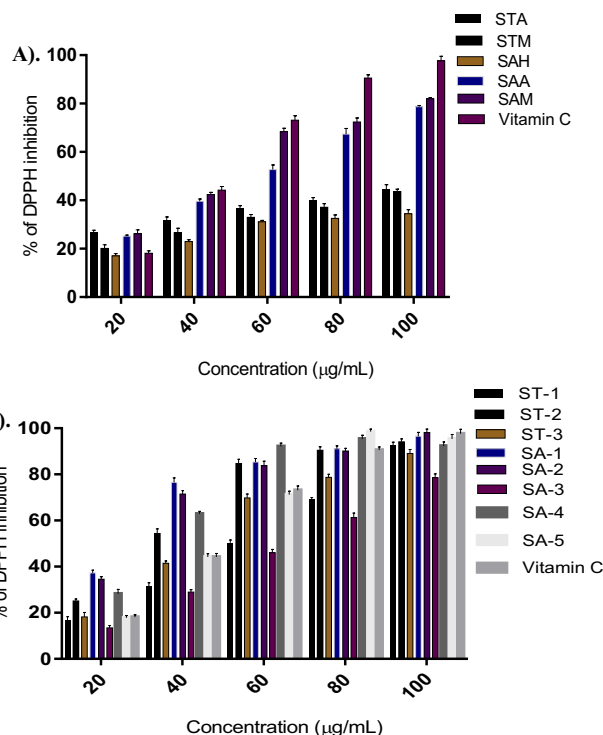


Fig. 2 (A) DPPH radical scavenging activity of different extracts; (B) isolates of *Shorea tumbergaia* (ST) and *Syzygium alternifolium* (SA) compared with standard Vitamin C.

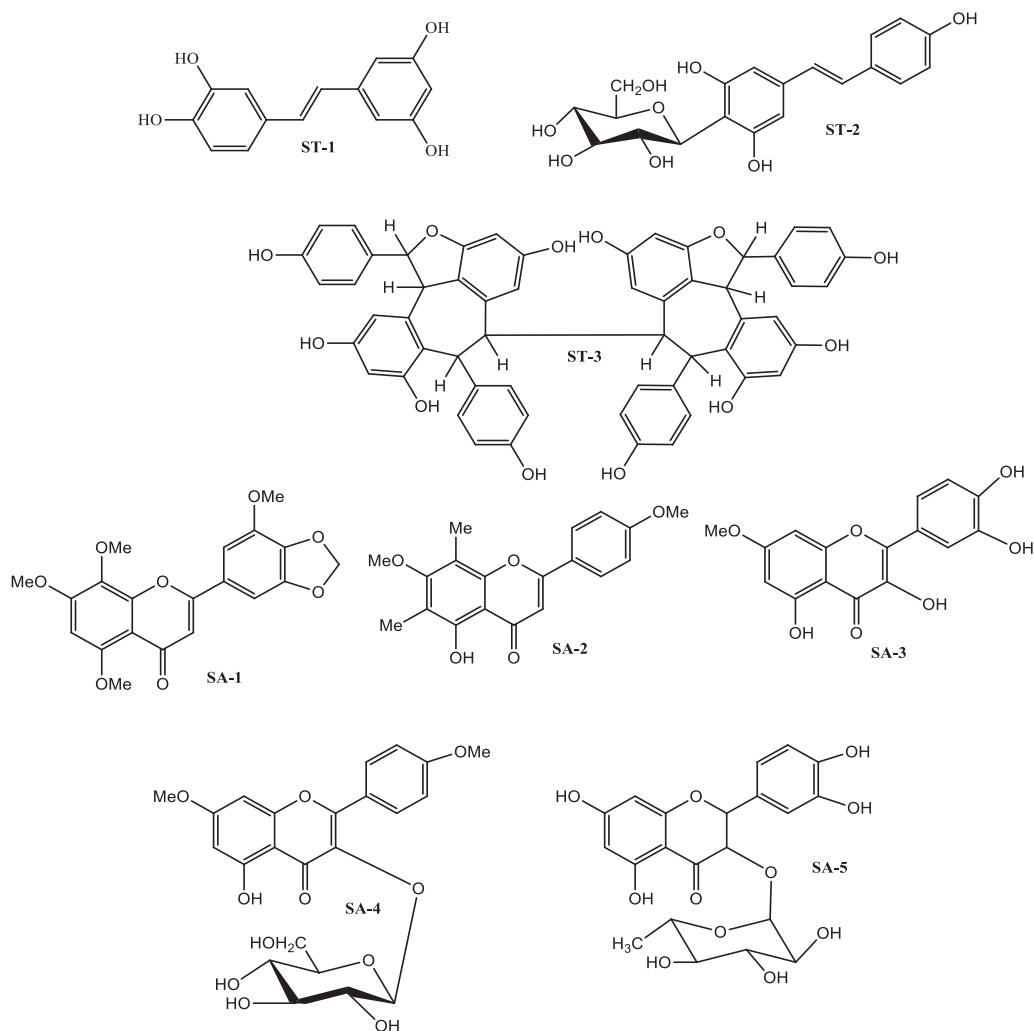


Fig. 3 Isolates from the stem bark of *Shorea tumbuggaia* (ST 1–3) and roots of *Syzygium alternifolium* (SA 1–5).

In the case of *S. alternifolium*, it was found that the acetone extract has the higher TPC (SAA, 84.39 ± 1.2 $\mu\text{g/mL}$ of GAEs), followed by methanol extract (SAM, 54.63 ± 1.2 $\mu\text{g/mL}$ of GAEs) and hexane extract (SAH, $44.33 \pm$

1.4 $\mu\text{g/mL}$ of GAEs). TFC of extracts of *S. tumbuggaia* and *S. alternifolium* is presented in Fig. 1, and measured in catechin equivalence (CE). Highest flavonoid content was found in SAA (132.28 ± 1.6 $\mu\text{g/mL}$) followed by STM (62.47 ± 1.1 $\mu\text{g/mL}$) whereas lowest content (10.21 ± 1.5 $\mu\text{g/mL}$) was found in STH. The extras STA, SAM and SAH showed moderate TFC results in 56.55 ± 1.2 , 56.45 ± 0.9 and 41.14 ± 1.6 $\mu\text{g/mL}$, respectively. Comparison of both plant extracts there is no correlation observed between TPC and TFC results. Among all extracts, STH extract showed least TP and TF contents, and hence did not consider for further work.

Table 1 Inhibitory concentration (IC_{50}) of isolated compounds of *Shorea tumbuggaia* and *Syzygium alternifolium* were computed with DPPH method.

S.No	Compound	IC_{50} ($\mu\text{g/mL}$)
1.	Ascorbic acid	45.97
2.	Picetannol (ST-1)	42.43
3.	Resveratrol-12-C- β -D-Glucopyranoside (ST-2)	70.47
4.	Hopeaphenol (ST-3)	60.16
5.	5,7,8,5'-tetramethoxy-3',4'-methylenedioxy flavone (SA-1)	100.65
6.	5-hydroxy-4',7-dimethoxy-6,8-di-C-methyl flavone (SA-2)	95.29
7.	Quercetin-7-O-methyl ether (SA-3)	37.52
8.	Kaempferol-7,4'-dimethylether-3-O- β -D-glucopyranoside (SA-4)	78.37
9.	Taxifolin-3-O- α -L-rhamnopyranoside (SA-5)	55.33

4.2. Free radical scavenging activities and isolation of bioactive compounds

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants (Cao et al. 1996). The radical-scavenging activities of the acetone and methanol extracts of the stem bark of *S. tumbuggaia* (STA & STM) and, *n*-hexane (SAH), acetone (SAA) and methanol (SAM) of *S. alternifolium* roots were estimated by comparing the percentage inhibition of formation of DPPH radicals with that of vitamin C shown

in Fig. 2A. The antioxidant potential of extracts in DPPH assay linearly correlated to its total phenolic compounds. It is found that the antioxidant activity of *S. alternifolium* root extracts increased proportionally to the phenolic content with a linear relationship between DPPH values. And the high level of antioxidant capacity is may be due to the maximum amount of total phenolic contents (TPC). In view of the presence of significant amount of phenolic contents and DPPH radical scavenging activities of extracts, except the *n*-hexane extract (STH) of *S. tumbergaia* remain all the five extracts were considered for further phytochemical examination individually.

The TPC result shows that the phenolic content present in acetone and methanol extracts (STA and STM) of *Shorea tumbergaia* are significant and almost similar. The TLC profile of both extracts was also found to be same and hence both extracts combined, and purification resulted in three compounds, piceatannol (ST-1), resveratrol-12-*C*- β -D-glucopyranoside (ST-2) and (+)-hopeaphenol (ST-3). Similarly, a systematic phytochemical examination of extracts of *Syzygium alternifolium* roots had resulted in five compounds, 5,7,8,5'-tetramethoxy-3',4'-methyleneoxyflavone (SA-1), 5-hydroxy-4',7-dimethoxy-6,8-di-*C*-methylflavone

(SA-2), quercetin 7-methylether (SA-3), kaempferol 7,4'-dimethylether 3-*O*- β -D-glucopyranoside (SA-4) and taxifolin 3-*O*- α -L-rhamnopyranoside (SA-5). The structures (Fig. 3) of the all the isolated compounds were established using mass, ¹H and ¹³C NMR spectral studies.

The antioxidant activity of the Isolates from *Shorea tumbergaia* (ST) and *Syzygium alternifolium* (SA) was evaluated by DPPH radical scavenging activity with vitamin C as standard as described earlier for the crude extracts (Vide supra), and the results were presented in Fig. 2B. The IC₅₀ values of the isolates from *Shorea tumbergaia* (ST) showed the highest antioxidant activity for ST-1 followed by ST-3 (tetrameric stilbene) and ST-2 (stilbene derivative) with IC₅₀ values 42.43, 60.16 and 70.47 in μ g/mL respectively. The antioxidant activity of piceatannol (ST-1) may be due to the existence of two phenol rings in conjugation with a double bond and ortho dihydroxy phenolic system. The significant antioxidant activity observed for hopeaphenol (ST-3) may be due to the presence of several phenolic moieties which gives stability for the molecular structure. The antioxidant activity associated with resveratrol glycoside (ST-2) may be due to the presence of ortho-dihydroxy system in the glycosidic portion of the

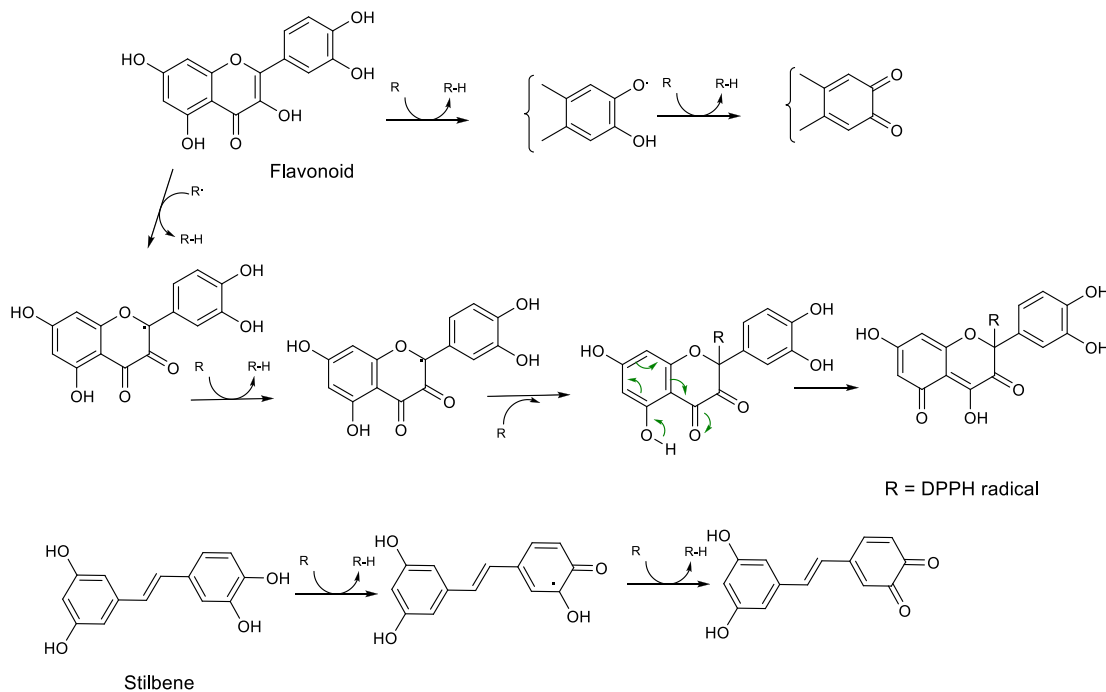


Fig. 4 The possible polyphenolic oxidation mechanism pathways by DPPH radical.

Table 2 Theoretical results of isolated compounds.

Compound	HOMO (eV)	LUMO (eV)	ΔE (eV)	IP (eV)	EA (eV)
ST-1	-5.54	-1.74	3.80	5.54	1.74
ST-2	-5.40	-0.57	4.83	5.40	0.57
ST-3	-5.36	-0.75	4.60	5.36	0.75
SA-1	-5.94	-1.76	4.17	5.94	1.76
SA-2	-5.91	-1.68	4.24	5.91	1.68
SA-3	-5.71	-1.79	3.92	5.71	1.79
SA-4	-6.22	-1.49	4.73	6.22	1.49
SA-5	-5.84	-1.51	4.33	5.84	1.51

Table 3 Binding interactions, binding energy, binding affinity, RMSD, strain and solvation of isolated flavonoids with SH3 domain of NADH.

Compounds	Interaction Protein–Ligand	Biding energy (kcal/mol)	Binding affinity (kcal/mol)	RMSD (Å)	Strain (kcal/mol)	Solvation (kcal/mol)
SA-1	Glu477—arene	−6.2	−6.1	0.6	3.5	−19.7
SA-2	Gu477—arene Lys502—arene	−6.5	−5.3	0.1	1.6	−17.9
SA-3	Glu477—arene Asp482—OH Ser499—OH	−6.2	−4.5	0.4	12.5	−15.5
SA-4	Ala470—OH Glu477—OH Glu477—OH Lys502—O Lys502—arene	−6.9	−5.4	0.7	34.2	−13.7
SA-5	Glu472—OH Glu474—OH	−5.8	−3.9	1.0	16.1	−13.1
ST-1	Pro473—OH Asp475—OH Glu477—arene Glu477—OH Phe478—arene Gln479—OH Lys500—OH	−6.6	−4.6	0.3	14.4	−21.0
ST-2	Glu469—OH Thr471—OH Asp475—OH Asn491—OH	−5.7	−4.1	1.2	29.0	−16.7
ST-3	Glu474—OH Asp475—OH Glu496—OH	−5.3	−2.0	0.1	13.5	−6.7

Table 4 Binding interactions, binding energy, binding affinity, RMSD, strain and solvation of isolated flavonoids with SPSB2.

Compounds	Interaction Protein–Ligand	Biding energy (kcal/mol)	Binding affinity (kcal/mol)	RMSD (Å)	Strain (kcal/mol)	Solvation (kcal/mol)
SA-1	Trp207—arene Gln209—O	−6.4	−4.9	0.5	3.8	−16.8
SA-2	Trp207—arene	−6.0	−4.4	0.3	2.5	−14.4
SA-3	Trp207—arene Trp207—OH	−6.4	−4.8	0.1	1.4	−16.8
SA-4	Pro70—OH Tyr20—O	−5.7	−4.7	0.7	11.1	−17.3
SA-5	Pro70—OH Gly208—OH	−5.7	−4.7	0.2	8.5	−17.3
ST-1	Pro70—OH Gly208—OH	−5.5	−5.0	0.3	3.6	−16.5
ST-2	Thr102—arene Val206—OH Gly208—OH	−5.7	−4.4	0.2	6.2	−14.6
ST-3	Val71—arene Val71—OH	−6.0	−6.5	0.5	9.4	−26.4

molecule. From the Table 1, it is evident that the flavonoids, SA-3 and SA-5, the isolates from *Syzygium alternifolium* (SA) showed outstanding antioxidant activities with IC₅₀ values (37.52 & 55.33 µg/mL) compared to that of other compounds. The possible polyphenolic oxidation mechanism pathways by DPPH radical shown in Fig. 4.

4.3. Theoretical and structure activity relationship studies

DFT studies were carried out to attain a better understanding of the antioxidant activity of isolated compounds of plants.

The HOMO, LUMO and MEP structures of all the isolated compounds were measured. In addition, the ability of compounds (ST 1–3 and SA 1–5) to transfer hydrogen was calculated in terms of electronic parameters and the results were shown in Table 2. Among the isolated plant phenolics, the compounds ST-1 and SA-3 were showed smallest HOMO-LUMO energy gap 3.80 and 3.92 eV, respectively. Furthermore, these two compounds have highest electron affinities 1.79 and 1.74 eV, respectively which representing its strongest potential as prominent antioxidants by single electron transfer reactions (in terms of hydrogen free radical

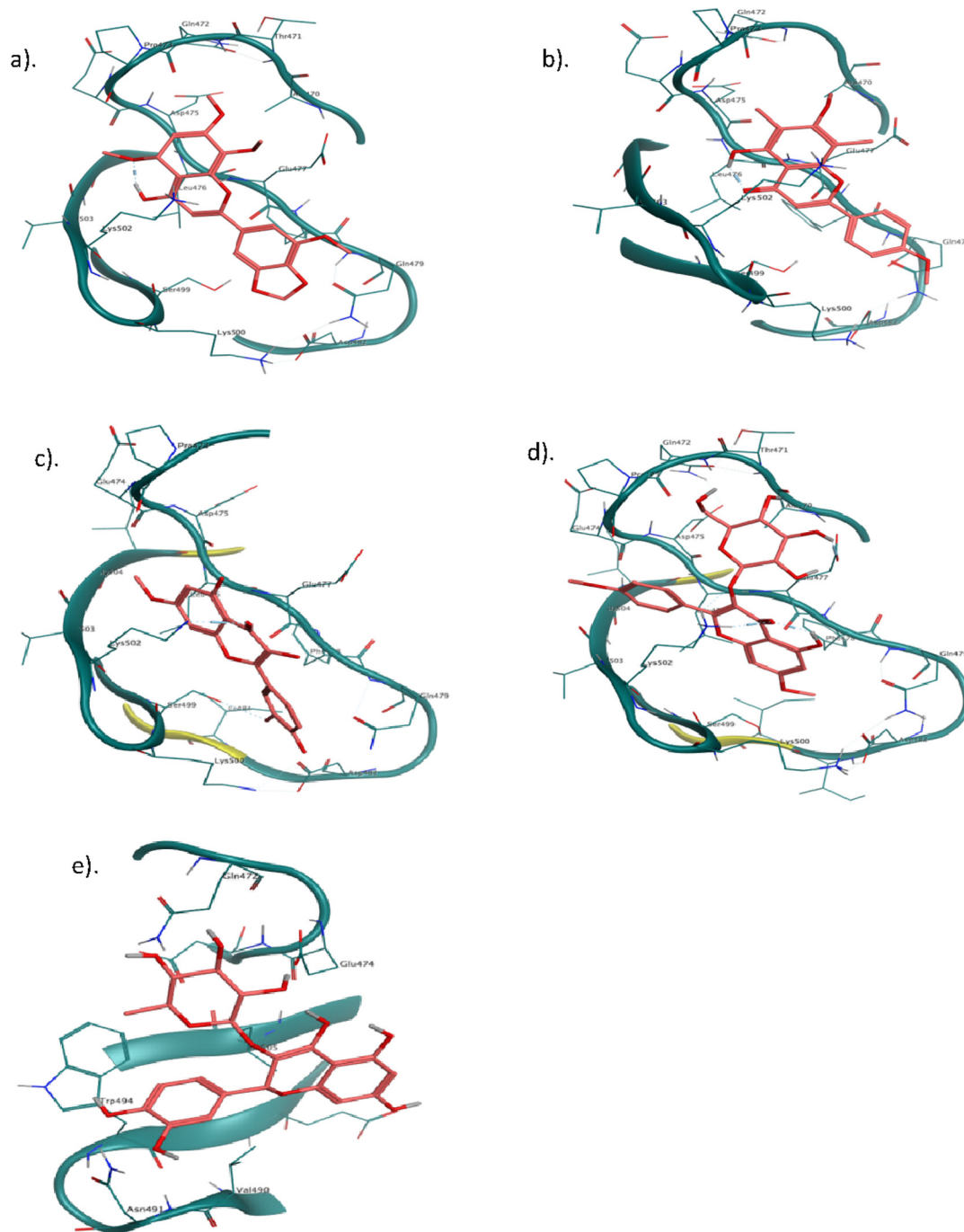


Fig. 5 Binding interactions of isolated compounds (a) SA-1, (b) SA-2, (c) SA-3, (d) SA-4 and (e) ST-1 with active site residues of SH3 domain of NADH.

donation). Also, SA-1 and SA-5 are the two another flavonoids have moderate antioxidant efficacy owing to finest HOMO-LUMO energy gaps 4.17 and 4.33 eV, respectively with significant electron affinities. Hence, the theoretical result showed that the general decreasing antioxidant trends as follows: ST-1 > SA-3 > SA-1 > SA-5 > SA-2 > ST-3 > SA-4 > ST-2.

4.4. Molecular docking results

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase possesses five cytosolic soluble SH3 domains, some of which implicated in the regulation of oxidase assembly and activity. Of these, Src homology 3 (SH3) domains is presented at the C terminal that specifically associated with PxxP motif of its partner protein as protein-protein interaction and activates the oxidases. Likewise, inducible nitric oxide synthase (iNOS) is one of the isoforms of nitric oxide synthase (NOS) that generates high level of endogenous nitric oxide (NO) during conversion of L-arginine to L-citrulline. SPRY domain is comprising SOCS box protein 2 (SPSB2) which act as negative regulator, binds with iNOS and maintain the lifetime and NO production. However, in this study, we conducted docking studies with SH3 domain and SPSB2 for isolated compounds. In addition, GB/VI interaction energies and binding affinities were calculated that reveal binding energy, binding affinity and critical interactions of leads molecules (Tables 3 and 4). Docking studies exhibited that SA-4, ST-1, SA-2, SA-1 and

SA-3 have shown eloquent binding energies of -6.9 kcal/mol, -6.6 kcal/mol, -6.5 kcal/mol, -6.2 kcal/mol and -6.2 kcal/mol and explicated critical interactions with pocket residues of SH3 domain, respectively. SA-4 displayed four bonds, arene-H bond and one polar bond between C ring and Lys502, glucopyranoside formed three bonds with Ala470 and Glu477. ST-1 is stilbenoid which displayed seven interactions, 3 and 4-dihydroxy phenol formed two bonds with Pro473 and Asp475, and one arene-H bond with Glu477. 3 and 5-dihydroxy phenyl exhibited four bonds such as 3-OH bond with Glu477, 5-OH bond with Gln479 and Lys500, and arene-H bond with Phe478. SA-2 formed two bonds such as C ring with Glu477 and A ring with Lys502. SA-1 formed one key arene bond via C ring with Glu477. SA-3 exhibited four bonds: A ring formed arene-H bond with Glu477, O of C ring bonds with Lys502, 3 OH and 4 OH of B ring bonds with Ser499 and Asp482 (Fig. 5). While, in case of SPSB2, SA-1 conferred two bonds such as C ring exhibits one arene-H bond with Trp207 and one bond with Gln209 (Fig. 6). SA-2 showed one arene-H bond via ring A with Trp207. SA-3 exhibited two arene-H bonds with Trp207. ST-3 displayed two bond interactions with Val71 and Gly208.

4.5. Bioavailability

The relationship between physicochemical and pharmacokinetic properties were measured using Lipinski rule of five (molecular weight < 1000, H-donor (< 10), H-acceptor (< 12) and cLogP

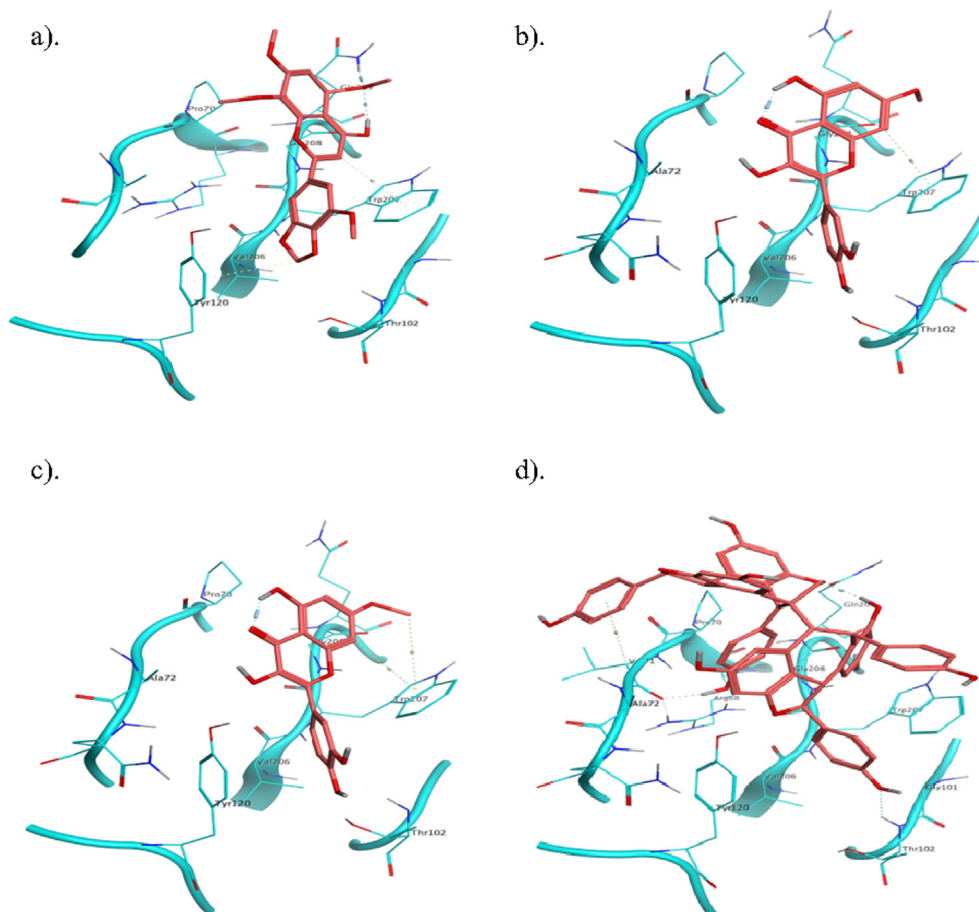


Fig. 6 Binding interactions of isolated compounds (a) SA-1, (b) SA-2, (c) SA-3 and (d) ST-3 with active site residues of SPSB2.

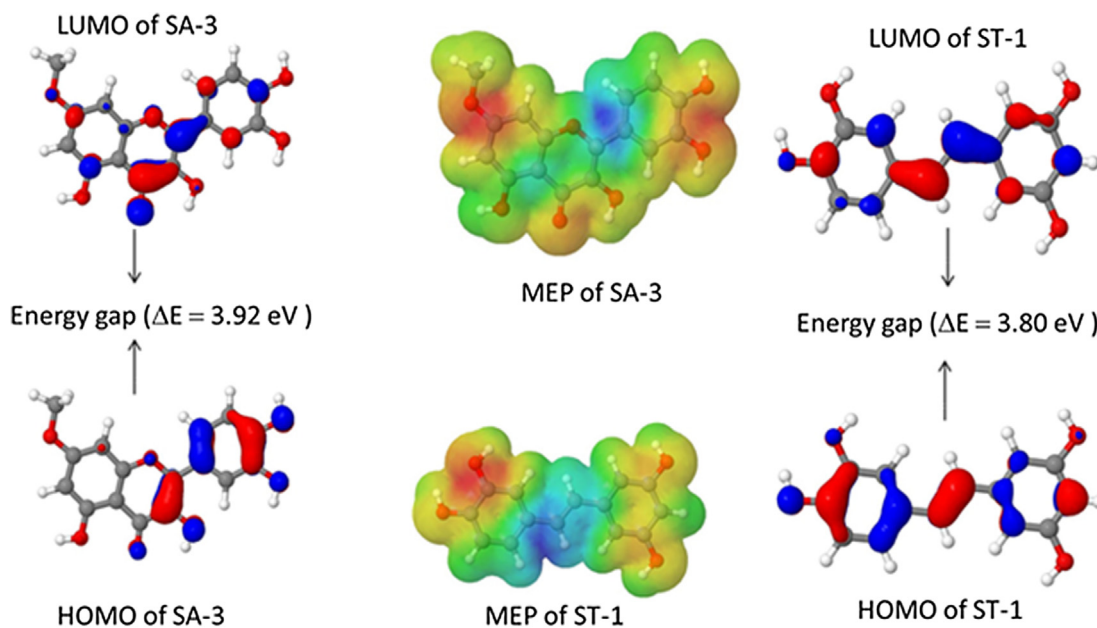


Fig. 7 Frontier molecular orbitals of SA-3 and ST-1.

(< 10) and the results were significant (Table S4). Lipinski rule of five revealed all the isolated plant phenolics showed molecular weight was found to be less than 500 (except ST-3) and LogP was found to be below five, hydrogen bond donors and hydrogen bond acceptors also were obeyed. These properties disclosed ADME properties that are beneficial to understand the drug mechanism within the cell environment, for instance, blood brain barrier permeability and interaction with different isoform cytochromes. Thus, the results of Lipinski rule recommended that these polyphenolics could be beneficial as antioxidant and endorsed for further *in vivo* studies.

5. Discussion

On comparison of Simulation results and the molecular docking parameters with the DPPH experimental results, the radical scavenging activities of isolated compounds were some deviations to the order in relation to hydrogen transfer due to several factors including solubility, polarizability, resonance effects and inductive effects. The capacity of polyphenolics to act as antioxidants depends on their molecular structure, O-H group in specific position on the flavonoid core and how fast it releases of hydrogen radical to involve in radical scavenging activity (Cai et al., 2014; Rice-Evans et al., 1996). The high antioxidant activity of the flavonoid (SA-3) and piceatannol (ST-1) may be attributed to the highly significant contribution of 3',4'- dihydroxyl groups on the B-ring as well as 3-hydroxy group on the C-ring in SA-3 (Rice-Evans et al., 1996) and the 3,4-dihydroxyl groups in conjugation of whole molecule in ST-1 (Piotrowska et al., 2012). In addition, it may thus be assumed that 3'-OH and 4'-OH are primarily involved in H-atom transfer reactions to DPPH in SA-3; and/or that each OH group strongly facilitates H-abstraction from the other by stabilizing the corresponding radical by a combination of electronic and H-bonding effects. This was further supported by the distribution of HOMO orbital in SA-3 (in ring-B and C) and ST-1 (Fig. 7), which denotes the strong antioxidant capabilities (Clare, 1995).

Free radical scavenging activity of dihydroflavonol glycoside i.e., taxifolin 3-O- α -L-rhamnopyranoside (SA-5) was lesser than that of SA-3 as the 3-hydroxyl group in SA-5 was blocked due to glycosylation. In addition, the double bond at C₂ and C₃ in the C ring is absent, and the protection of the hydroxyl group(s) with sugar moieties are responsible for the lesser free radical-scavenging activity than SA-3 (Burda and Oleszek, 2001). This is further evidenced by the distribution of HOMO orbital only on ring B of SA-5. In case of tetrameric stilbenes ST-3, there is no specific theoretical parameter which supports the experimental results, but it is assumed that the significant antioxidant is may be due to polyphenolic moieties and the stable molecular structure. The less potent antioxidant activity of SA-4, SA-1 and SA-2 in comparison with that of SA-3 and SA-5 must be associated with the absence of ortho-dihydroxyl and 3-OH groups in the former three flavonoid derivatives. These three compounds (SA-4, SA-1 and SA-2) exhibited moderate activities as free-radical scavengers in the DPPH assay, in comparison with reference antioxidant (vitamin C) because of the presence of double bond at C₂ and C₃ in the C ring and polyoxygenated flavonoid moiety/moieties which have capability to bind free radicals.

6. Conclusions

The two isolates viz., quercetin 7-O-methyl ether (SA-3) and piceatannol (ST-1), showed potent antioxidant properties. Whereas the isolates taxifolin-3-O- α -L-rhamnopyranoside (SA-5) and hopeaphenol (ST-3), showed moderate radical scavenging activity when compared to the other isolates. Hence, the two isolated plant phenolics SA-3 and ST-1, can be explored for their applications in the prevention of free radical related diseases viz., hypertension, anti-inflammatory, cancer, rheumatoid arthritis, diabetes, vascular diseases and hyperplastic disease associated with oxidative damage.

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2019.12.017>.

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