Chapter 2

Heterocyclic Compounds and Their Biological Applications of *Semecarpus anacardium* L.f

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Abstract

Heterocyclic compounds such as (1) 1H-Pyrazole, (2) 2, 6-Piperidinedione, (3) 13-Tetrade-cene-1-ol acetate, (4) 2-Methyl-3-(4-methoxybenzoyl)indole, (5) 5H-Naphtho [1,8-bc] thiophen-5-one]; Essential fatty acids and lipid-soluble bioactives, Glycolipids i.e. Monogala-ctosyldiacylglycerol (MGDG), Digalactosyldiacylglycerol (DGDG), Sulfoquinovosyldiacyl-glycerol (SQD), Steryl glucoside(SG), Acylated steryl glucoside (ASG)], Amino acids i.e. tryptophan, thiamine, riboflavine, histidine, nicotinic acid & Tocopherols [α, β and γ-Tocopherols], Flavanoids and Biflavanoids have been isolated in our laboratory from the *Semecarpus anacardium* L.f Nuts, Leaves, Flowers, Stem bark and Root bark. These were characterized through their chemical and spectral data. These were nutritionally considered as a new non-conventional supply for pharmaceutical industries and edible purposes. The knowledge concerning the composition and properties of *Semecarpus anacardium* L.f would assist majorly in efforts of nutritional and industrial, applications of this plant. To explore the binding mode and understanding of key active site residues of the extracted natural compounds such as fatty acids in the active site, docking studies were carried out by taking the crystal structure of human FAAH (PDB ID: 3K84). The Docking studies indicated the presence of 20(Amino acids) amino acids in the active site. Phytocomponents in the extract imply the phytopharmaceutical importance of the plant. Most of the chemical compounds can target both gram-positive and gram-negative bacteria, Anti-cancer activity, Radical Scavenging activity is being reported for the first time.

Keywords

Semecarpus anacardium L.f, Anacardiaceae Family, Glycolipids, Amino Acids, Tocopherols, Biflavonoids (Heterocyclic Compounds), Antimicrobial Activity, Anti-cancer Activity and Anti Oxidant Activity

2.1 Introduction

Semecrpus anacardium (SA) L.f (Family: Anacardiaceae) [1-2], Trees, up to 25 m height with grey bark exfoliating in small irregular flakes, leaves simple alternate, obviate-oblong, flowers are greenish white, in panicles and nuts is about 2.5 cm long, shining black when ripe, seated on an orange-colored receptacle form of the disk. The black corrosive juice of the pericarp contains tarry oil consisting of 90% of oxy-acid anacardic acid & 10% of higher nonvolatile alcohol called cardol, also contains catechol and a mono-hydroxy phenol called as anacardol. the most significant components of the *Semecarpus anacardium* L.f oil are phenolic compounds [3], on exposure to air, Phenolic compounds get oxidized to quinines, the oxidation process can be prevented by keeping the oil under nitrogen, is well known for its viceant liquid which causes severe burns and allergic edema in exposed parts is obviously due to the lipoid-soluble C_{15} chain present in the catechol, and it finds frequent application in Indian medicine in the treatment of gout, rheumatic pains and other ailments [4]. Hetero cyclic compounds are essential to life. These are present in a wide variety of many natural products of plant and marine origin. During our literature survey we found that, the Anacardiaceae family has 77 genera and 850 species, and contains several anticancer drugs isolated from Anacardiaceae family. We have selected *Semecarpus anacardium* L.f for its high medicinal value in ayurvedic and siddha systems and isolated several active constituents in our laboratory. These were isolated, purified and highly analyzed basing on FT-IR, ^{1}H NMR, ^{13}C NMR, MASS, NP-HPLC, GC & GC-MS Analysis.

2.2 Materials and Methods

2.2.1 Plant Material

All parts like Flowers, Leaves, Stem bark, Nuts, Root bark, were collected from field area nearer to the village Nandgaon which is situated at nearly 20-25 km. outskirts to Kolhapur city, Maharashtra, India (Figure 1). All plant material specimen's were identified by Dr Vatsavaya S. Raju, Former Head and Chairperson-BOS Plant Systematic Laboratory, Department of Botany, Kakatiya University, Warangal (A. P.), India and conformed as *Semecarpus anacardium* L.f. (syn: *Anacardium latifolium* Lam., *A. orientale* Steud.) of Anacardiaceae Family and plant specimen deposited at Kakatiya University Herbarium, Warangal (KUW) with accession number 1874. It is locally known as '*nalla jeedi*' and popularly known '*markingnut/dhobinut.*

Figure 1. *Images of plant material collection.*

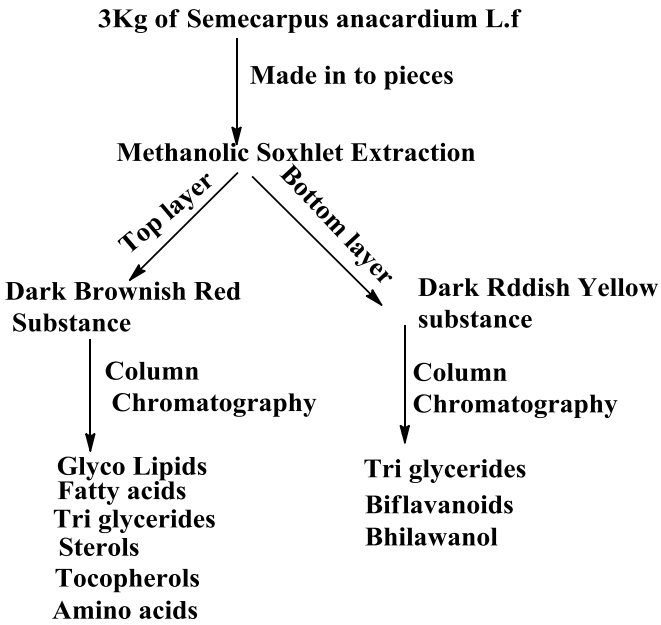


Figure 2. *Scheme of separation of compounds from Semecarpus anacardium* L.f.

2.2.2 Preparation of Plant Extract

Isolation: *Semecarpus anacardium* L.f. 3 kg Fruiting Nuts were extracted with 3 lit of methanol by soxhlet extraction for 72 hours, two layers observed dark brownish black substance at top layer and dark reddish yellow at bottom layer, These were separated by separating funnel and were concentrated by vacuum-evaporation and weighed. The crude extract of the two layers were subjected for column chromatography followed by TLC, Prep. TLC & by individual crystallization. Compounds were isolated, purified and highly analyzed basing on FT-IR, ^{1}H NMR, ^{13}C NMR, MASS, NP-HPLC, GC & GC-MS Analysis. Top layer contains, Triglycerides, sterols, Glycolipids, Tocopherols, Aminoacids and bottom layer contains, Triglycerides, Biflavonoids, and Bhilawanol (Figure 2).

2.3 Glycolipids

2.3.1 Column Chromatography and Thin-Layer Chromatography of Lipid Classes

The Total lipids were separated into different classes by elution with solvents of increasing polarity over a column packed with silica gel (100-200 mesh), the major portion of Glico lipids were eluted with excess volume of acetone, the composition was determined by GC/FID. By means of TLC on Silica gel plates a further characterization of Glico lipids were done, each spot was identified with lipid standards as well as their reported retention factor (Rf) values. Individual bands were visualized under UV light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v), Monogalacto-syldiacylglycerol(MGDG), Digalactosyldiacylglycerol (DGDG), Sulfo - quinovosyldiacylglycerol (SQD), Steryl gluco side (SG), Acylated steryl glucoside (ASG) were obtained from *Semecarpus anacardium* L.f Nuts [5] (Figure 3).

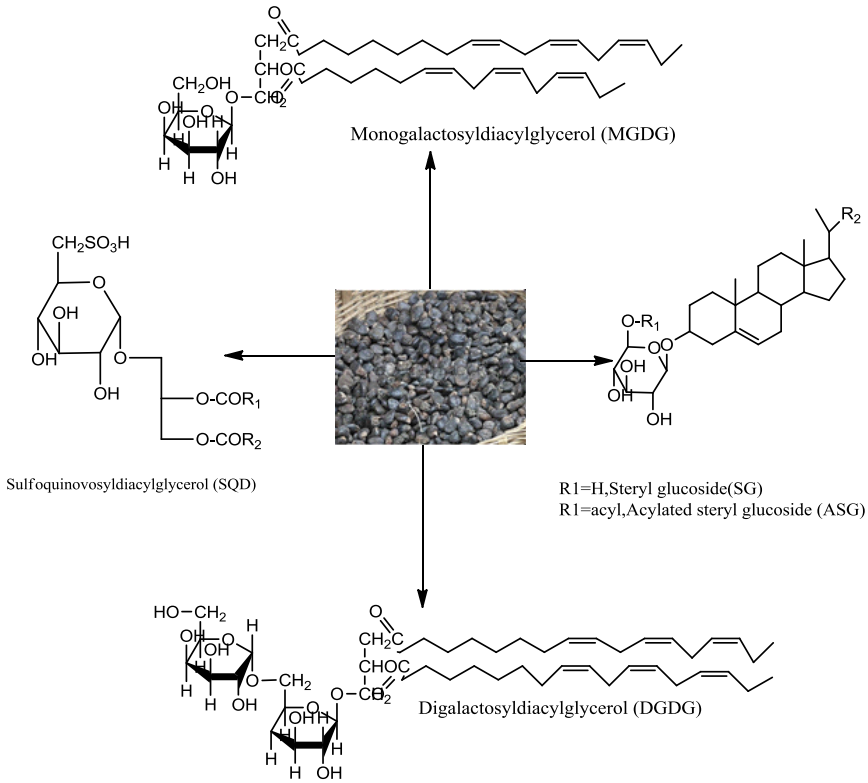


Figure 3. *Glycolipids from Semecarpus anacardium* L.f *Nuts.*

2.3.2 Tocopherols

Normal phase high performance liquid chromatography (NP-HPLC) analysis of tocopherols: NP-HPLC has advantage of allowing the resolution of the four isomers (α, β, γ, δ), RP-HPLC does not allow the complete resolution of β & γ isomers, and consequently, in RP-HPLC, these two vitamins were quantified together and also NP-HPLC was selected to avoid extra sample treatment (e.g., saponification), The analysis was performed with a solvent delivery LC-9A HPLC [6]. The chromatographic system included a model 87.00 variable wavelength detector and a 250×4 mm i.d. LiChrospher-Si 60, 5 μm column. The separations of tocopherols were based on isocratic elution when the solvent flow

rate was maintained at 1 mL min^{-1} at a column back-pressure of about 65-70 bar. The solvent system selected for elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. Twenty µL of the diluted solution of TL in the mobile phase were directly injected into the HPLC. Tocopherols were identified by comparing their retention times with those of authentic samples (Figure 4).

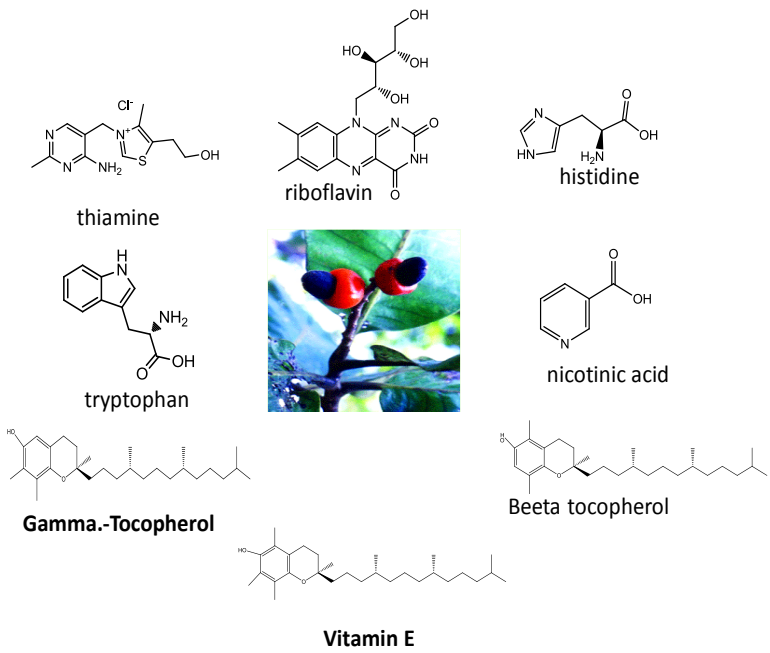


Figure 4. *Amino Acids and Tocopherols from SA Fruiting Nuts.*

RP-HPLC with UV-detector on a Lichrosolv C-18 [7] column with methanol in Na-Phosphate buffer gradient elution used For Amino acids. Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. The 20 amino acids that are found within proteins convey a vast array of chemical versatility. The 10 amino acids that we can produce are

alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. Tyrosine is produced from phenylalanine, so if the diet is deficient in phenylalanine, tyrosine will be required as well. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Humans can produce 10 of the 20 amino acids. The others must be supplied in the food.

To explore the binding mode and understanding of key active site residues of the extracted natural compounds such as fatty acids in the active site docking studies were carried out by taking the crystal structure of human FAAH (PDB ID: 3K84). Docking studies indicated the presence of 20 (Amino acids) amino acids in the active site.

2.4 Heterocyclic Compounds from SA Flowers & Leaves

Semecarpus anacardium L.f. Shade dried Leaves, Flowering Buds 3 kg were extracted with 4 lit. of each polar and non polar solvent by soxhlet extraction for 72 hours. These extracts were concentrated and analyzed additionally by using Gas Chromatography-Mass Spectrometry. GC-MS analysis of phyto constituents in plants gives a clear picture of the pharmaceutical value of that plant (Figure 5 & Figure 6). The mass spectrum of the hexane extract of *Semecarpus anacardium* L.f. was compared with the available library sources (NIST08 LIB, WILEY8 LIB) it was found that *Semecarpus anacardium* Flowers revealed the presence of 1H-Pyrazole2.15%, γ-Tocopherol 0.96%, γ-Tocopherol1.33%, Vitamin E2.5% 2,6-Piperidinedione 2.14% and Leaves contains 2-Methyl-3-(4-methoxybenzoyl) indole 0.22%, 5H-Naphtho [1,8-bc] thiophen-5-one1.59%, γ-Tocopherol1.68% & Vitamin E2.88% (See Table 1 & 2) & (Figure 7).

Table 1. *Heterocyclic compounds and their medicinal properties from Semecarpus anacardium* L.f *Flowers.*

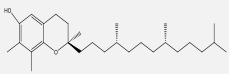
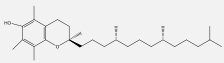
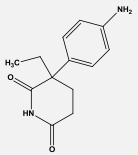
S.NO	Chemical Name	Chemical Structure	Chemical Formula	Molecular Weight
1	1H-Pyrazole		$C_3H_4N_2$	68.0773
2	γ-Tocopherol both leaves and flowers		$C_{28}H_{48}O_2$	416.68
3	Vitamin E Both leaves and flowers		$C_{29}H_{50}O_2$	430.71
4	2,6-Piperidinedione		$C_{13}H_{16}N_2O_2$	232.3

Table 1. *Continued.*

S.NO	R.T/Min	Area %	Medicinal Uses
1	16.44	2.15	Anti inflammatory, anti diabetic and antibacterial activity [8].
2	24.55	0.96	Preventing diseases of the heart and blood vessels including hardening of the arteries, heart attack, chest pain, leg pain due to blocked arteries, and high blood pressure [9].
3	25.18	2.55	Breast cancer, and breast cysts preventing diseases of the heart and blood vessels including hardening of the arteries, heart attack, chest pain, leg pain due to blocked arteries, and high blood pressure [9].
4	29.46	2.14	Immunomodulatory agent with antineoplastic and antimitogenic properties [10].

Table 2. *Heterocyclic Compounds and Their Medicinal Properties from Semecarpus Anacardium* L.f *Leaves.*

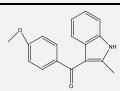
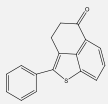
S.NO	Chemical Name of the Compound	Chemical Structure	Chemical Formula	Molecular Weight
1	2-Methyl-3-(4-methoxybenzoyl)indole		$C_{17}H_{15}NO_2$	265.3116
2	5H-Naphtho[1,8-bc]thiophen-5-one		$C_{17}H_{12}OS$	264.05

Table 2. *Continued.*

S.NO	R.T/Min	AREA %	Medicinal Uses
1	19.879	0.22	It is used in the study of CB_2 mediated responses and has been used to investigate the possible role of CB_2 receptors in the brain [11].
2	21.926	1.59	Anti inflammatory, anti diabetic and antibacterial activity [12].

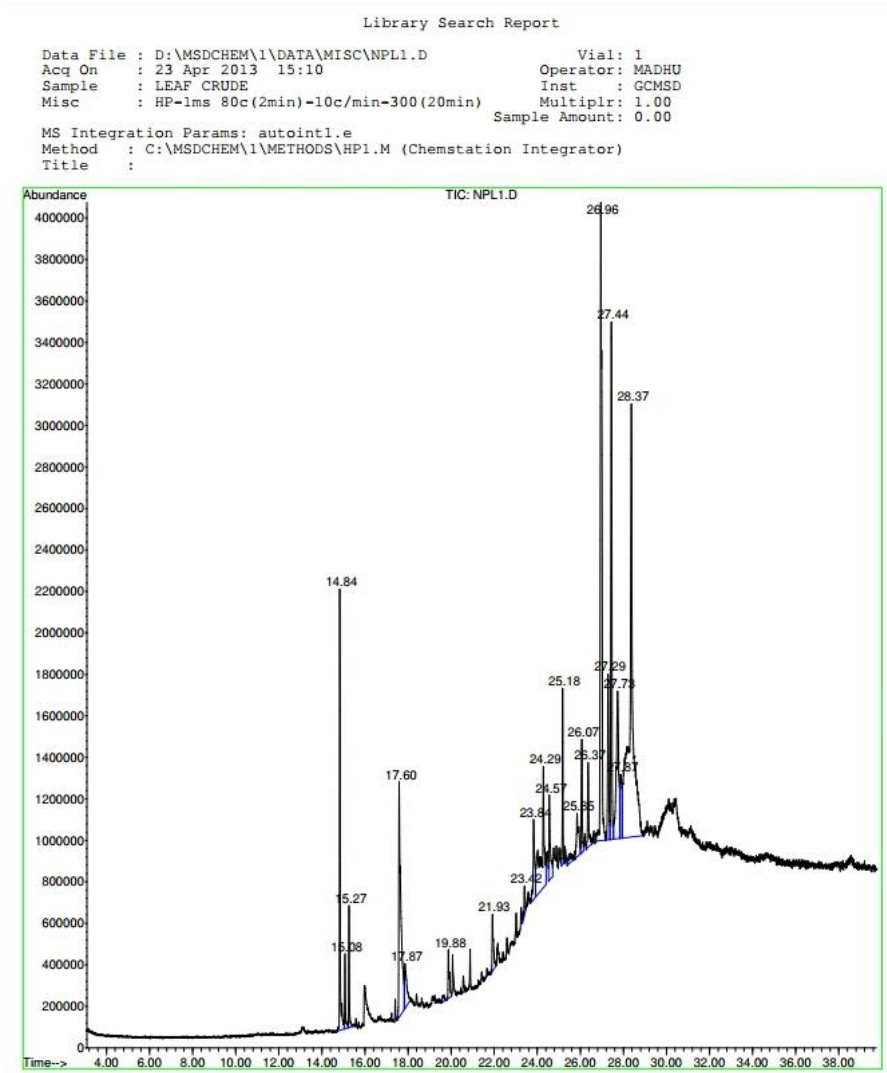


Figure 5. *GCMS reports of SA Leaves & Flowers (1).*

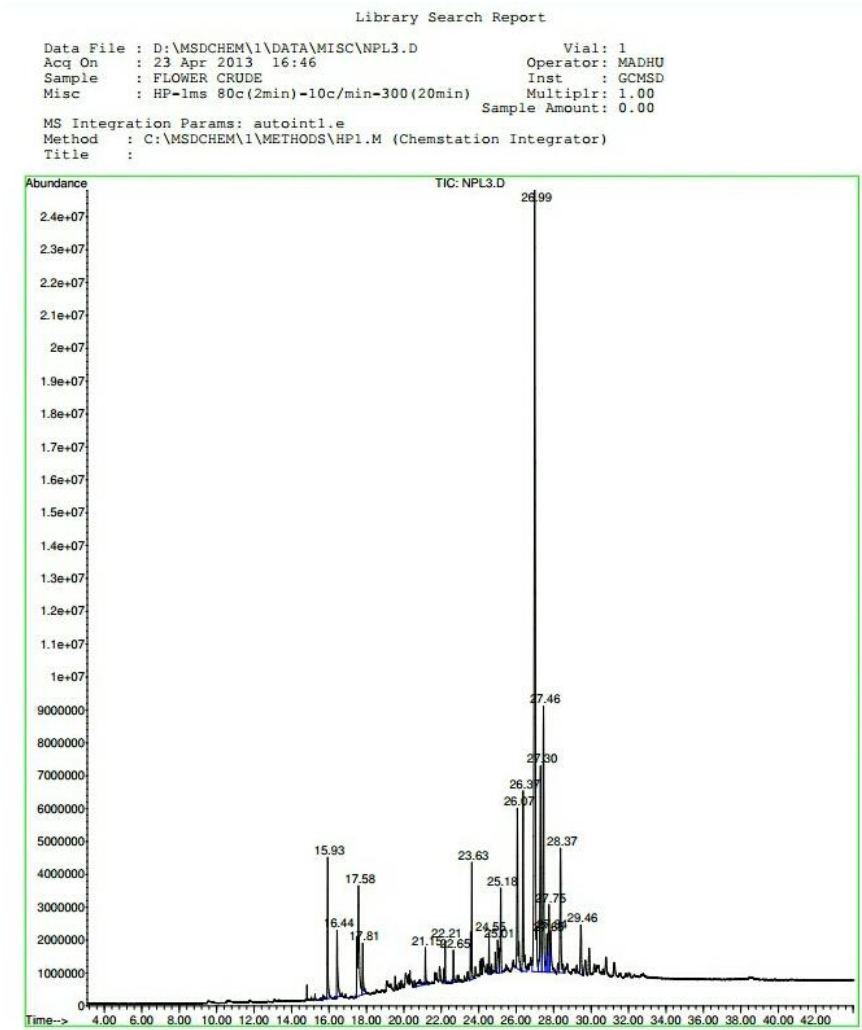


Figure 6. *GCMS reports of SA Leaves & Flowers (2).*

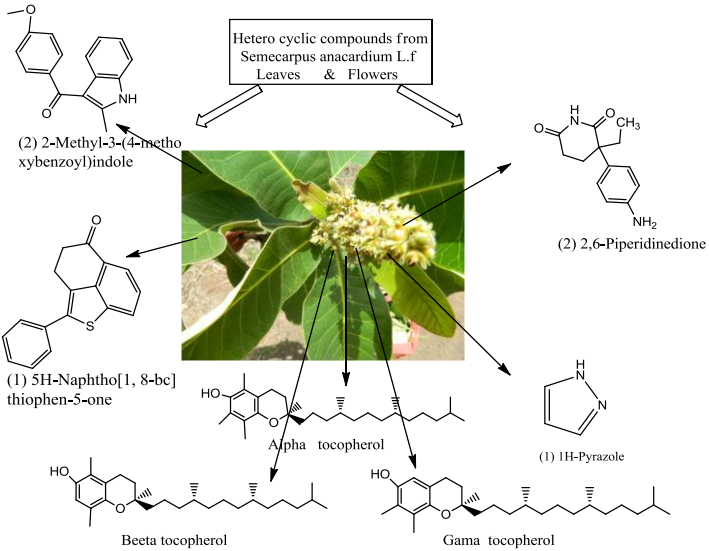


Figure 7. *Heterocyclic compounds from SA Leaves & Flowers.*

2.5 Isolation and Purification of Biflavonoids from SA Nuts, Leaves and Root Bark

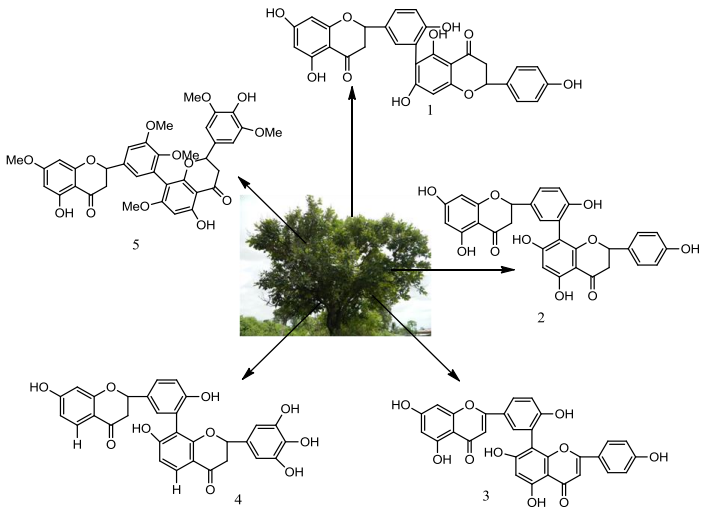


Figure 8. *The various compounds isolated from Semecarpus anacardium*
1. Tetrahydrorubustaflavone, 2. Tetrahydroamentoflavanone, 3. Amentoflavone, 4. Semecarpu flavanone, 5. Nallaflavanone.

Semecarpus anacardium L.f. 3 kg nuts were collected and shade dried, The nuts were directly percolated with cold petroleum ether 5-6 times, filtered and concentrated by vacuum evaporation. Then the nuts were made in to small pieces and percolated with petroleum ether 5-6 times, filtered and concentrated by vacuum evaporation. Again the powdered nuts were percolated with hot petroleum ether 5-6 times, filtered, and concentrated by vacuum evaporation.

After that, the same powdered nuts were extracted with cold acetone by changing the solvent for 3 hours in three intervals, filtered and concentrated by vacuum evaporation.

After cold acetone percolation, nuts were subjected to hot acetone extract, and concentrated by vacuum evaporation.

The above fourth concentrate was then fractionated using silica gel column with appropriate solvent gradient (Benzene: Acetone 9:1) and the Biflavanoids obtained from the fraction of 13 (Benzene: Acetone; 9:1) were rechromatographed and purified. Leaves and Root bark were extracted individually with 4 lit. of each polar and non polar solvents by soxhlet extraction for 72 hours, was concentrated by vacuum-evaporation, and submitted for spectral analysis, Spray reagents were used for the detection of Biflavonoides Thin layer plates Ceric sulphate (70% in con H_2SO_4) Ref: cambie and James, 1967, Kawano et., al.1964. Biflavonoids gave a greenish-violet ferric reaction, A pinkish-red color with sodium borohydride -hydrochloric acid, Mg-Hcl and an Orange-red color with $NaBH_4$-Hcl, Spectral data of compounds 1-5 were consistent with the reported literature, Therefore, the structure of these compounds were determined as follows:

Compound 1: It appears as a yellow needles, mp 251 ℃, Its UV absorption in methanol are at λmax (nm) 289, 224 and 211, its IR absorptions shows at Hydroxyl (3394 cm^{-1}), conjugated carbonyl (1643), and aromatic rings

(1597, 1516, 1493 and 1458 cm^{-1}, The negative ESI-MS at m/z 541[M-H]$^-$, Thus the molecular formula was deduced to be $C_{30}H_{22}O_{10}$, ^{1}H NMR (Acetone d6, 400 MHz): δ 12.38 (1H, *s*, OH), 12.16 (1H, *s*, OH), 9.62 (1H, *br s*, OH), 7.35 (2H), 7.29 (1H, *m*), 7.13 (1H, *d*, *J* =2.3 Hz, H-2'), 6.88(1H, *d*, *J* = 8.4 Hz, H-5'), 6.81 (2H), 6.04 (1H, *s*), 5.89 (1H), 5.88 (1H), 5.47 (1H), 5.43 (1H), 3.32 *(*1H), 3.26(1H), 2.72 (1H), 2.66 (1H).

^{13}CNMR (acetone-*d6*): CH_2(43), CH(127.4), CH(126.3), CH(116.1), CH(120.1)

CH(127.4), CH(128.3), CH(116.1), CH(95.1), C(131.1), C(115), C(95.8), C(102.8), C(101.9), C(157), C(164), C(160.2), C(116), C(196.3), CH(82), CH(82.8), C(163.6), C(162.5) δppm. Spectral data of compounds 1 were consistent with the reported literature, Therefore, the structure of compound 1 was determined to be as tetra hydrorobustaflavone.

Compound 2: It appears as yellow amparphous substance, mp 235 ℃. Its UV absorption in methanol are at λmax (nm) 285, 225(sh), 330(sh)., its IR absorptions shows at Hydroxyl group 3323.88, benzene rings at 1636.74, 1344.05, 1219.65, 772.64. The negative ESI-MS at m/z 541.1[M-H]$^-$, Thus the molecular formula to be $C_{30}H_{22}O_{10}$, ^{1}H NMR (Acetone d6, 400 MHz): δ 12.28 (1H, s, OH), 12.17(1H, s, OH), 7.21 (4H, m), 6.85 (1H, d, *J* = 8.19), 6.71 (2H, d, *J* = 8.04), 6.05 (1H, s), 5.88 (2H, s), 5.44 (2H, m), 3.16 (2H, brm), 2.77 (2H, br m).

^{13}CNMR (acetone-*d6*): CH_2 (43.46), CH(120), CH(120), CH(128.3), CH(116.1), CH(95.1), CH(95.6), CH(94.6), C(130.9), C(131.2), C(105.9), C(102.8),

C(101.6), C(157.4), C(162.7), C(160.2), C(16 4.7), C(163.8), C(196.8), CH(82.8), C(163.6), C(155.9) δppm. Spectral data of compounds 2 was consistent with the reported literature. Therefore, the structure of compound 2 was determined to be as tetrahydroroamento flavone.

Compound 3: It appears as yellow cubes, mp 235℃, UV λmax (nm) 285, 223(sh), 330(sh). The positive ESI-MS at m/z 538.46, its molecular formula is $C_{30}H_{18}O_{10}$, its IR absorptions shows at Hydroxyl group 3400, benzene rings at 1605, 1445, 1310, 1235, 1148, 1078, 820, ^{1}H NMR (Acetone d6, 400 MHz) δ 12.28 (1H, s, OH), 12.17(1H, s, OH), 7.21 (4H, m), 6.85 (1H, d, J = 8.19), 6.71 (2H, d, J = 8.04), 6.05 (1H, s), 5.88 (2H, s), 5.44 (2H, m), 3.16 (2H, brm), 2.77 (2H, br m), ^{13}CNMR (acetone-*d6*): CH_2 (43), CH(126.3), CH(120.1), CH(120), CH(128.3), CH(116.2), CH(114.2), CH(95.1), CH(95.6), CH (94.6), C(131.1), C(131.2), C(105.1), C(102.8), C(101.6), C(164.9), C(146.2), C(145.9), C(160.2), C(164.77), C(163.8), C(196.8), C(196.8), CH(82.8), CH (83.1), C(163.6), C(155.9) δppm. Spectral data of compounds 3 was consistent with the reported literature, Therefore, the structure of compound 3was determined to be as amentoflavone.

Compound 4: It appears as yellow amarphous substance, mp 249℃, UV λmax (nm) 291, 296(sh), The positive ESI-MS at m/z 543.2, so the molecular formula is$C_{30}H_{22}O_{10}$, its IR absorptions shows at Hydroxy291, 296(sh), 338(sh), ^{1}H NMR (Acetone d6, 400 MHz) δ3.08 (2H, m, trans), 5.42 (2H, d d, J = 4.0, 12.0Hz), 7.15 (l H, *d, J*= 8.5 Hz), 7.37 (l H, *d, J* = 2.0 Hz) and 7.46 (l H, *d d, J= 2.0, 8.5* Hz), at 6.34 (l H, *d, J* = 8.0 Hz), 6.14 (l H, *d d, J= 2.0, 8.0* Hz) and 6.22 (l H, *d, J = 2.0* Hz), 6.84 *(d, J = 2.0* Hz), 6.92 *(d, J= 2.0* Hz), 7.26 (2H, s), 7.64 (2H, s), 7.76 (l H, s) and 8.50 (l H, s), 6.52 (l H, *d, J* = 8.0 Hz) and 6.72(l H, *d, J* = 8.0 Hz). ^{13}CNMR (acetone-*d6*): CH_2(42.7), CH(126.3), CH(120.1), CH(109.2), CH(128.3), CH(106.8), CH(107.4), CH(106.8), CH(102), CH(129.7), CH(130.8), C(131.2), C(136), C(114.3), C(115.1), C(113.3), C(113.4), C(163.5), C(146.4), C(160.2), C(164.4), C(146.4), C(134.9), C(190.9), CH(82.8), CH(83.4), C(164), C(153.9) δppm. Spectral data of compounds 4 was consistent with the reported literature, Therefore, the structure of compound 4 was determined to be as Semecarpuflavanone.

Compound 5: It appears as yellow amarphous substance, mp 249 ℃, UV λmax (nm) 296, 296(sh), 338(sh). The positive ESI-MS at m/z 674.65, so the molecular formula is $C_{36}H_{34}O_{13}$, Its IR absorptions shows at Hydroxylgroup (3450.12); broad), methoxy groups(2830), Chelated flavanonecarbonyl

(1650) and benzene rings(1590.9). ^{1}H NMR (Acetone d6, 400 MHz) δ 2.78(dd,2H, J=4,17H, cis-protons), 5.32(dd,2H, J=4,12Hz), 6.10(d, 1H, J=2Hz), 6.18(d, 1H, J=2Hz), 6.78(d, 1H, J=2Hz)6.90(d, 1H, J=2Hz), 7.30(d, 1H, J=2Hz), 7.40(d, 1H, J=2Hz), 3.48, 14.28(s, 1H) and 14.44(s, 1H), 8.74(s,1H). ^{13}CNMR (acetone-*d6*): OH(5.35), CH(5.51), CH(6.22), CH(6.18), CH(6.24), CH(6.99), CH(6.53), CH(7.37), CH_2(3.38), CH_3(3.83),

CH_3(55.8), CH_3(56.1), CH_3(60.6), CH_3(56.1), CH_2(43), CH(120), CH(102.1), CH(108.3), CH(94), CH(94.8), C(134.4), C(135.2), C(126.8), C(102.4), C(101.3), C(162.3), C(163.4), C(136.3), C(165.8),

C(147.6), C(154), C(164.1), C(151.1), C(147.6), C(196.8), CH(83.1), CH(83.4), C(163.2), C(158.3) δppm. Spectral data of compounds 5 was consistent with the reported literature, Therefore, the structure of compound 5 was determined to be as Nallaflavanone.

2.6 Docking Studies

In the present study, the molecular docking study has been conducted with 5 Biflavonoids on PTB1B targets. It also regulates the hepatocyte growth factor receptor signaling pathway through dephosphorylation of MET. From the docking studies, we have explored different probable binding pockets, putative active site residues, binding modes of extracted natural compounds from plants in different targets according to their mechanism of action. The molecular docking studies could provide substantial design clues for the development of novel, potent inhibitors for PTBIB targets. All computations and molecular

modeling studies were carried out on Schrodinger software. A dataset comprising of 5 Biflavonoids were drawn in ChemDraw and converted into 3D-molecules with all possible tautomers and chiral centers. The converted 3D-molecules were minimized with OPLS-2005 force field using water as solvent in the GB/SA continuum solvation model. The probable binding modes of best docked compounds are shown in Figures 9-13 and its interaction profile is shown in Table 3. The docking parameters and physicochemical properties of Biflavonoids in PTP1B targets are shown in Table 4.

2.6.1 SCF in the Active Site of PtP1B

The two hydroxyl groups in catechol moiety showed hydrogen bond interactions with Asp236. The hydroxyl group of hydroxyl chromanone ring connected to catechol interacts with Glu200. The carbonyl group of dihydroxy chromanone ring showed hydrogen bond interaction with Asn193 and hydroxyl group's hydrogen bond interaction with Ser190 and Glu276. BF1 showed hydrophobic interactions with Phe196, Ile281, Phe280, Leu192 and Ala189.

2.6.2 THAF in the Active Site of PtP1B

The two hydroxyl groups in catechol moiety showed hydrogen bond interactions with Asp236. The hydroxyl group of hydroxyl chromanone ring connected to catechol interacts with Glu200. The carbonyl group of dihydroxy chromanone ring showed hydrogen bond interaction with Asn193 and hydroxyl groups hydrogen bond interaction with Ser190 and Glu276. PBF1 showed hydrophobic interactions with Phe196, Ile281, Phe280, Leu192 and Ala189.

Table 3. *The key active site residues in PTP1B targets around 5Å.*

Target	Active site (5Å)
PTP1B	SER187, PRO188, ALA189, LEU192, ASN193, LEU195, PHE196, LYS197, GLU200, LEU232, GLU276, GLY277, ALA278, LYS279, PHE280, ILE281 and MET282

Table 4. *The docking parameters and physicochemical properties of flavonoids in PtpIB target.*

Flavonoid	gscore	evdw	ecoul	energy	emodel	Mol.wt	logP	PSA
SCF	-7.42	-39.36	-11.14	-50.51	-62.3	542.5	1.7	197.14
THAF	-6.9	-39.86	-4.08	-43.94	-61.18	542.5	2.76	193.26
NF	-6.59	-34.83	-5.88	-40.71	-59.33	674.66	5.46	172.91
A	-6	-33.03	-13.07	-46.1	-64.47	538.47	2.62	192.59
THRF	-5.68	-45.04	-7.88	-52.91	-63.71	542.5	3.13	194.07

3D Structures of Biflavonoids from SA Nuts, Leaves and Root Bark:

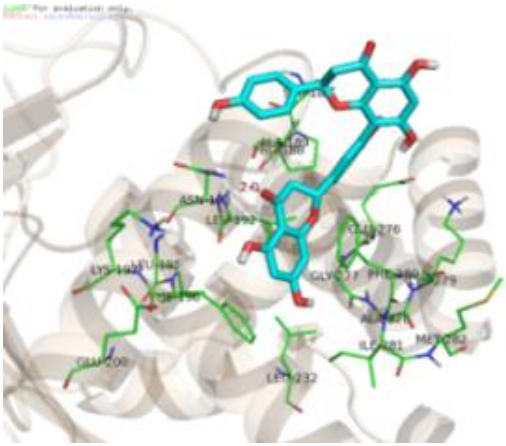


Figure 9. *THRF.*

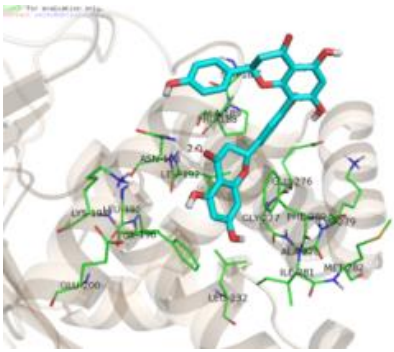


Figure 10. *THAF.*

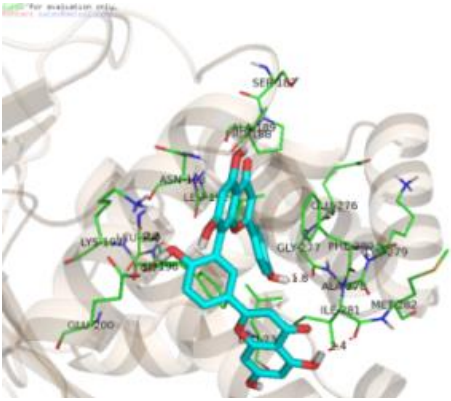


Figure 11. *AF.*

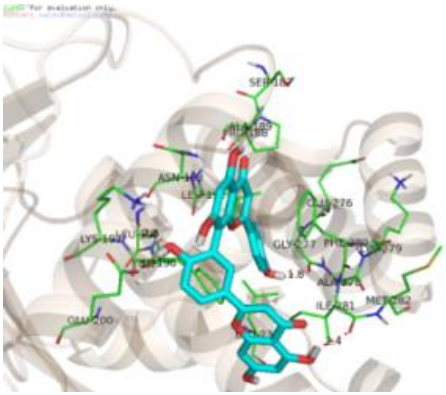


Figure 12. *SCF.*

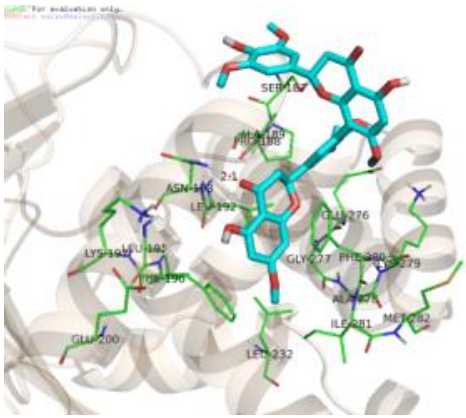


Figure 13. *NF.*

2.7 Antimicrobial Activity of the Isolated Compounds

The antimicrobial activity of the isolated compounds and their derivatives were determined by using well diffusion method against different pathogenic reference strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic bacteria and *Candida* reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media Petri plates using a cork borer and the isolated compound and their derivatives at a dose range of 300 - 1.4 µg $well^{-1}$ was added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Neomycin and Miconazole at a dose range of 300-1.4 µg $well^{-1}$ and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 30 ℃ and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration.

Table 5. *Anti microbial Activity of Biflavonoids.*

COMPOUNDS	Staphylococcus aureus MTCC 96	Klebsiella planticola MTCC 530	Bacillus subtilis MTCC 121	S.aureus MLS16 MTCC 2940
THRF	9.37	18.75	9.37	9.37
THAF	18.75	9.37	9.37	9.37
AF	9.37	18.75	18.75	--
SCF	9.25	--	18.75	--
NF	18.75	--	--	18.75
Neomycin	18.75	18.75	18.75	18.75
Miconazole	--	--	--	--

Table 5. *Continued*

COMPOUNDS	Micrococcus luteus MTCC 2470	Escherechia coli MTCC 739	Pseudomonas aeruginosa MTCC 2453	Candida albicans MTCC 3017
THRF	18.75	--	9.37	18.77
THAF	9.37	--	9.37	18.77
AF	9.37	9.37	18.75	9.37
SCF	9.37	0	9.75	18.75
NF	--	9.75	9.75	--
Neomycin	18.75	18.75	18.75	--
Miconazole	--	--	--	9.37

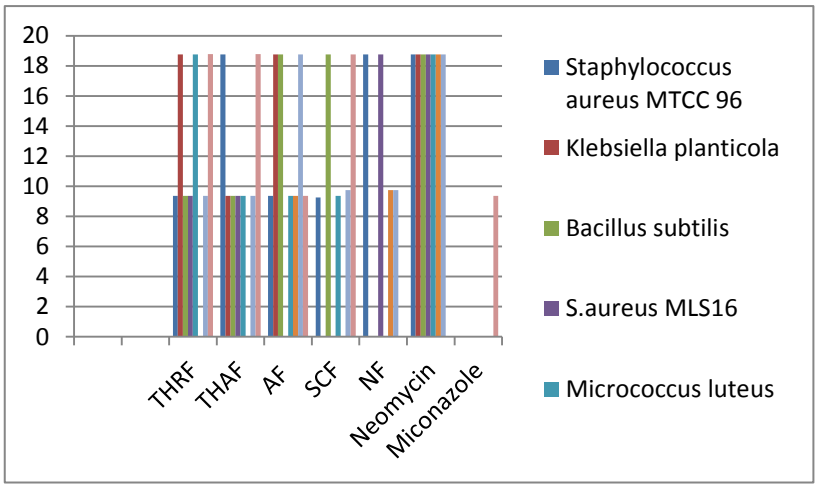


Figure 14. *Graphical representation of Antimicrobial activity of Biflavonoids from SA.*

2.8 Radical Scavenging Activity Using DPPH Method

The free radical scavenging power of the extracts was determined by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging method. Aliquots of 0.2 ml of the extracts (1 mM) were mixed with 2 ml of 0.1 mM methanolic DPPH. The volume was made up to 3 ml with methanol. The solutions were incubated in dark at room temperature for 40 min. Absorbance was read at 517 nm using methanol as a blank and methanolic DPPH as control. Methanolic solution of tert-butyl hydroxy anisole (BHA) at 1 mM was taken as reference. The scavenging activity was calculated using the following equation:

(%) Free radical scavenging activity = Absorbance of DPPH - Absorbance of sample X 100

Table 6. *Radical Scavenging Activity of Biflavonoids from SA.*

Sample Name	% Free radical Scavenging activity
BHA(1Mm)Reference antioxidant.	93.467
THRF	72
THAF	72
AF	70
SCF	71
NF	65

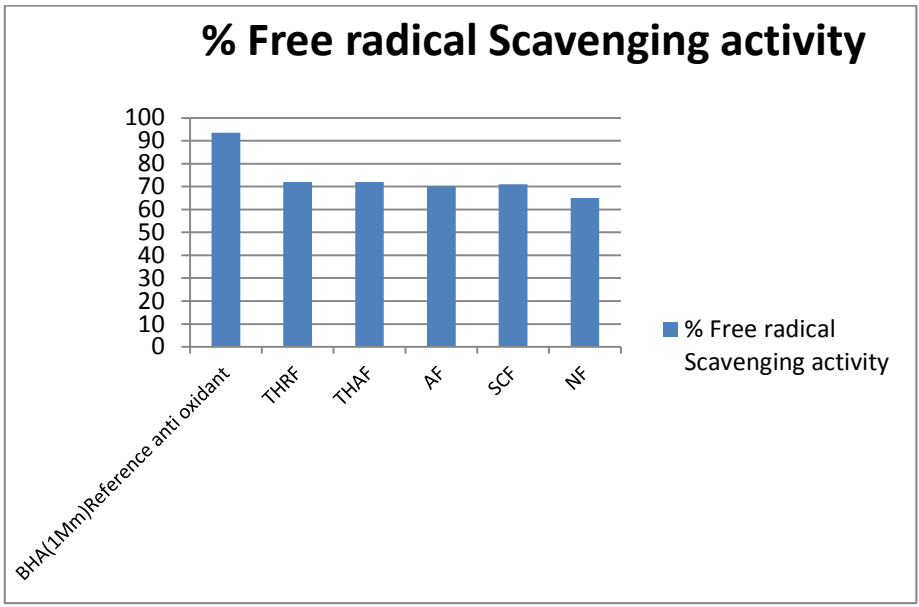


Figure 15. *Graphical representation of Radical Scavenging activity of Biflavonoids from SA.*

2.9 Conclusion

The present study is the first report on the *Semecarpus anacardium* L.f Nuts, Leaves & Flowers which is a good source of essential fatty acids and lipid-soluble bioactivities. Amino acids & Tocopherols were nutritionally considered as a new non-conventional source to supply for pharmaceutical industries and edible purposes. Tocopherols also have great utility in preserving the taste and preventing the oxidation or rancidity of many foods that contain oils and fats. Docking studies indicated the presence of 20 (Amino acids) amino acids in the active site. Phyto components present in the extract shows the phyto pharmaceutical importance of the plant [13-16]. To explore the binding mode and understanding of key active site residues of the extracted natural compounds such as fatty acids in the active site docking studies were carried out by taking the crystal structure of human FAAH (PDB ID: 3K84) [17] Most of

the chemical compounds isolated from Semecarpus anacardium can target both gram-positive and gram-negative bacteria, Anti cancer activity, Radical Scavenging activity.

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