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Research Article

CORRELATION BETWEEN METAL IONS AND ORGANIC COMPOUNDS FROM SEMECARPUS ANACARDUM L.F., THEIR BIOLOGICAL ASPECTS AND DOCKING STUDIES

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ABSTRACT

Medicinal plants after Really, have been playing an important role in the survival of the ethnic communities, natural drugs have recently been received greater importance all over the world, medicinal plants have also responded on the changing environmental conditions. Medicinal plants produce specific secondary metabolites, which can detoxify some of toxic metals. Many metals are essential micronutrients that play a crucial role in certain enzymes used for proper functioning of the body. The lack of proper micronutrients causes health problems in many undeveloped nations, particularly in children and pregnant women. Therefore, it is very important for understanding their nutritive and medicinal value. We have selected *Semecarpus anacardium* L.f belonging to, Anacardiaceae family which has 77 genera and 850 species frequently found in dry lands rather than in damp localities and commonly known as Ballataka or Bhilwa. For its high medicinal value in ayurvedic and siddha systems, we have selected this and isolated several active chemical constituents in our laboratory. The plant is a good source of minerals like Calcium (Ca),Magnesium(Mg), Potassium(K), Manganese(Mn), Sodium(Na), Lead(Pb), Boran(B), Sodium(Na), Zink(Zn), Nickel(Ni), Aluminum(Al) and this is the first time report on whole plant extracts and ash contents, The structural elucidation and pharmaceutical properties of some of these compounds are being presented in this paper.

INTRODUCTION

Semecarpus anacardium L.f Suppl P.I(1918) and Sing NP(1998) seed, the 'Marking Nut' is used in Ayurveda for the treatment of rheumatoid arthritis, gout and other inflammatory diseases, tumors, asthma, epilepsy, psoriasis and leprosy. The antimicrobial activity of the methanolic Nuts, Flowers, leaf, Root bark, Stem bark extract of *Semecarpus anacardium* was evaluated against *both gram +ve and gram –ve bacteria.* The methanolic extracts of Leaf, Flowers, Stem bark, Root bark, Nuts extracts of this plant were also subjected to evaluation of antioxidant activity by using DPPH free radical scavenging method and also Nitric oxide radical scavenging method. The preliminary photochemical studies on *Semecarpus anacardium* showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums. Further fluorescent analysis of different soxhlet extracts and whole plant powders provided the additional support for the qualitative chemical analysis findings. The results suggest that the Nuts of Semecarpus anacardium has photochemical Prakash Rao *et.al*., (1973), Gedam PH *et.al*.,(1974), Ishatulla.K .*et.al*.,(1977), Murthy SSN (1983)[6,7],(1984)[8],(1998), (1992)[10],(1987)[11]. properties and may be used for curing various ailments. Various functional groups were present in the crude powder and extract of Semecarpus anacardium Leaves, Flowers, Root bark, Stem bark, Nuts, were identified using FT-IR spectrometry. Our results suggested that, The FT-IR analysis of leaf, Flowers, Root bark, Stem bark, and Nuts powder of Semecarpus anacardium proved the presence of alcohols, phenols, alkanes, alkenes, carboxylic acids, ethers, esters, aliphatic iodo compounds and polysulfides. The above FT-IR studies of Semecarpus anacardium Leaves, Flowers, Root ark, Stem bark, Nuts revealed the different characteristic peak values with various functional groups present in them. Therefore, the objective of the present study is to determine the various metal ions and Organic compounds Present in the whole plant of *Semecarpus anacardium* L.f by using nitric acid digestion procedure with ICP-AES technique.

MATERIALS AND METHODS

Semecarpus anacardium L.f. nuts were collected from field area very nearer to the village Nandgaon, Kolhapur City,

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Maharashtra, India. All plant material specimen's were identified by Dr Vatsavaya S. Raju, Plant Systematic Laboratory, Department of Botany, Kakatiya University, Warangal (A. P. State) and conformed it as *Semecarpus anacardium* L.f. (syn: *Anacardium latifolium* Lam., *A. orientale* Steud.) of Anacardiaceae and plant specimen deposited at Kakatiya University Herbarium, Warangal (KUW) with accession number1874. It is locally known as 'nalla jeedi' and popularly known 'marking nut/dhobi nut (Fig.1-3)

Fig .1 Plant material collection and experimental images.

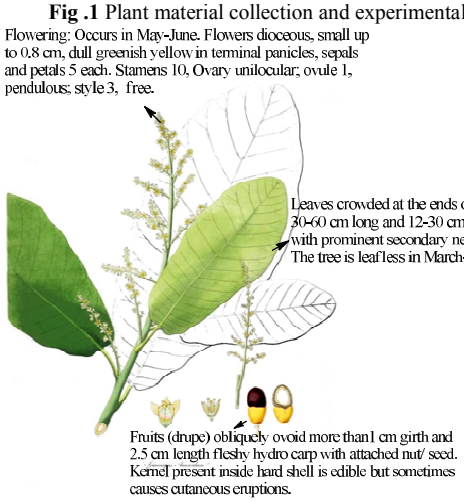


Fig .2 Morphology of the plant

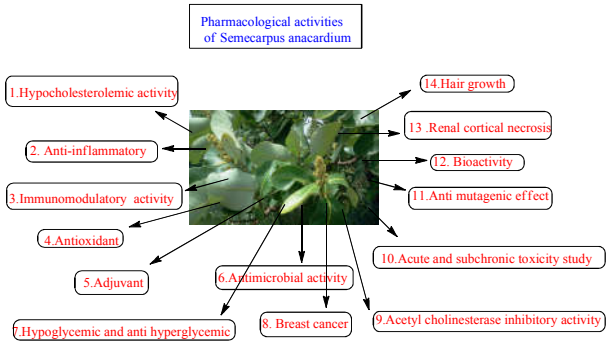


Fig 3 Pharmalogical aspects of SA L.f

1.Hypocholesterolemic activity[12], 2.Anti-inflammatory[13], 3.Immunomodulatory activity[14] 4.Antioxidant[15], 5.Adjuvant[16], 6.Antimicrobial activity[17], 7.Hypoglycemic and anti hyperglycemic[18] 8. Breast cancer [19], 9. Acetyl cholinesterase inhibitory activity [20], 10. Acute and subchronic toxicity study Choudhari CV. *et.al*., (2007), 11. Anti mutagenic effect Prabhu. D. *et.al*., (2005), 12. Bioactivity Krishna Rajua.AV.*et.al*(2005), 13. Renal cortical necrosis Matthai. TP (1979), 14.Hair growth Semalty.M. *et.al*., (2008).

Extraction and purification

All the plant material *S.A* L. f. were collected and shade dried. 3 kg Nuts, 7kg Leaves, 3Kg flowers, 3kg stem bark and 10kg root bark were collected individually and dried under shade. After shade drying, each one of them was powdered and finally got 2 kg of leaves powder, 1.5kg of flowers powder, 2Kg of stem bark powder and 3kg of root bark powder were obtained. These powders were extracted each with 3 Liters of hexane/methanol by Soxhlet extraction method for 72 hours. The excess of solvent from crude extracts was distilled off and the crude was weighed. The individual weight of the extracts was subjected for column chromatography. The individual fractions were purified by crystallization.

Determination of metal ions in Plant Extracts: 1 gm of the plant sample by weight was taken in a beaker and 5 ml of 70%concentrated nitric acid was added and kept on hot plate at 85° C for 15 mins. Subsequently, it was cooled and then made to 50 ml with the help of distilled water. Blank samples were also processed and analyzed simultaneously. The solutions were then analyzed with an inductively coupled plasma atomic emission spectrometer.

Determination of metal ions in Ash content: Shade dried leaves power6.8114 g , taken in silica crucible and kept in muffle in furnace at 550^0c for 5 hours white ash was obtained with 6.5319gm , Shade dried flowers were taken 1.7664gm and heated in furnace at 550^0c for 5 hours white ash was obtained with 1.666 gm ,Shade dried stem bark powder taken 7.6625gm and heated in the furnace at 550^0c for 5 hours white ash was obtained with 6.5563gm,Shade dried root bark power taken 7.5725gm and heated in the furnace at 550^0c for 5 hours white ash was obtained with 6.4568gm , 15.45gm of Nuts were taken and made in to ash 0.6116gm.To determine the total concentration of metal ions in plants was used by wet digestion. Weighed 1 g of each plant part ash was dissolved in 10 ml of warm 20%(v/v) *HCl* in distilled water and whose volume was made to 50 ml .The filtrate was used to test the presence of elements ICP-OES Limmatvapirat.C, *et.al*., (2012), Pdnekar, *et.al*., (2013) was used to determine several trace metal concentration. The instrument was calibrated and standardized with different working standards and the concentrations of metals in each sample were measured individually.

ICAP 6500 - A powerful system Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is a fast, multi-elemental technique used to measure trace metals such as lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn) and major cation's such as calcium (Ca), magnesium (Mg) and sodium (Na). Inductively Coupled Plasma techniques operate by decomposing a liquid sample by intense heat into a cloud of hot gases with an Inductive coupled plasma (a state of matter containing electrons and ionised atoms of Argon). The plasma reaches temperatures of around 10,000°C. The high

temperature causes excitation and ionisation of the sample atoms. Once the atoms or ions are in their excited energy states, they can decay to lower energy states whilst emitting light of specific wavelengths depending on the elements in the solution. In OES, the intensity of the light emitted at specific wavelength calibration is necessary for quantitative analysis (Fig. 4-7).

Fig. 4 ICAP 6500 DUO

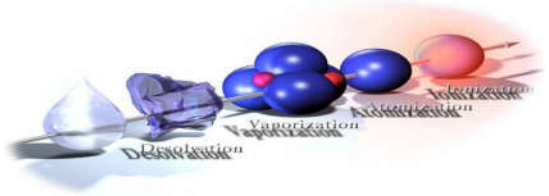


Fig 5 Inductively Coupled Plasma Atomic Emission Spectroeter

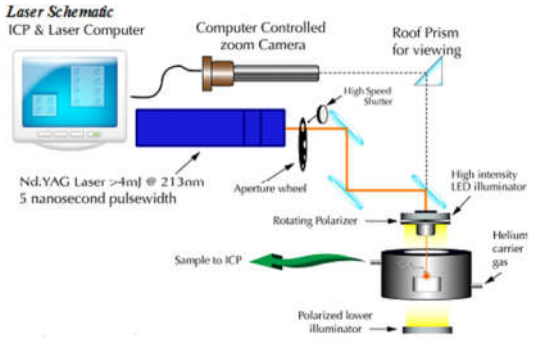


Fig.6 Inductively Coupled Plasma Atomic Emission Spectrometer

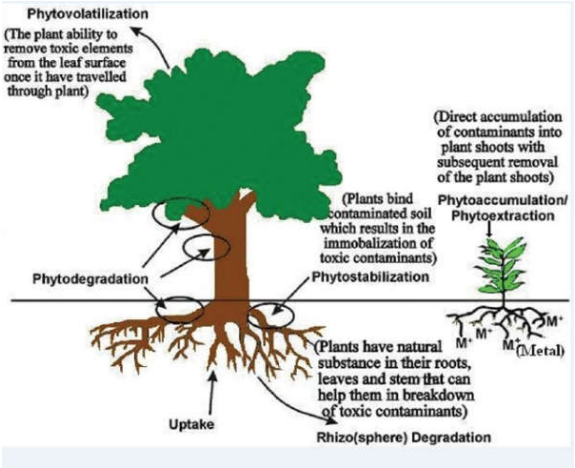


Fig.7 Transition mechanism in plants for metal accumulation.

By comparing the intensity of light emitted by solutions of known metal concentrations with unknown sample solutions, metal concentration can be determined. Lengths is measured and used to determine the concentrations of the elements of interest. Atomic Emission Spectroscopy (AES or OES) uses quantitative measurement of the optical emission from excited atoms to determine analyte concentration, Analyte atoms in solution are aspirated into the excitation region where they are dissolved, vaporized, and atomised by a plasma(Fig,8).

The instrumental parameters and operating conditions for ICP-AES

Model: ICAP 6500Series Duo: Thermo scientific, Argon gas used, ICAP 6000Series (ICP Spectrometer)

Model: ICAP 6500Series Duo	Instrument parameter
Soft ware's used ITEVA A NALYST	Optics temp 38^0
Camera temp -45^0C	Rf Power 1300 w,
Plasma (Ionized Argon gas)	Pump rates 40 rpm
SAMPLE CODE: SA Methanolic Root Bark	Aux gas flow 0.5 Lit/min,
	Neb gas flow 0.5Lit/min,
	Coolant gas flow 12 Lit/min,
	gas Flow normal

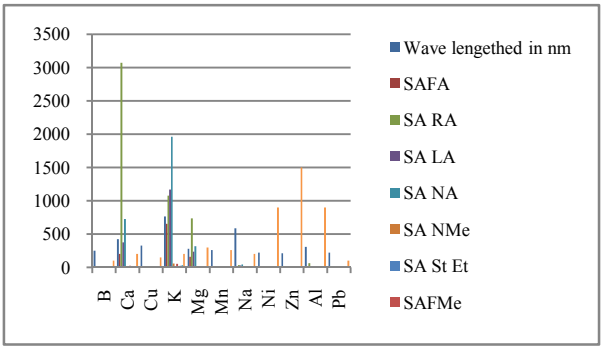


Fig.8 Graphical representation of multi metals present in the extracts and ash content from SA with their permissible limits.

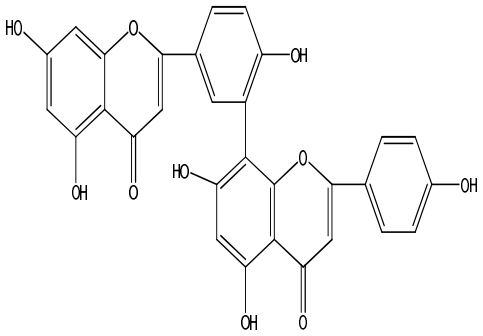
Determination of Organic compounds (Biflavanoids):

Isolation and Purification: Isolation and purification of Biflavonoids were differ from those of other natural products which were generally isolated by sequential extraction with solvents of increasing polarity and the appropriate fractions were purified by usual chromatographic techniques such as column chromatography and thin layer chromatography (TLC) Compounds were isolated, purified and highly analyzed basing on FT-IR, ^{1}H NMR,^{13}C NMR,MASS ,NP-HPLC,GC & GC-MS Analysis. Biflavanoids have been isolated from the acetone soluble fraction of Leaf extract and an ethanolic extract of the defatted nut shells of *Semecarpus anacadium* L.f .

Biflavanoids 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.

Compound: 1 Amento Flavone

Compound 1 was obtained as a yellow powder. The Mg-HCl reaction was positive, which confirmed that **1** was a flavone. Its UV absorptions in methanol are at UV (MeOH) $_{max}$ (log) 335.0 (2.26), 2 12.0 (3.15) nm; m/z: 538.09, It's molecular formula$C_{30}H_{18}O_{10}$,



Compound :1

IR (KBr): $_{max}$ 3435-3100 (broad), 1730, 1654, 1579, 1492, 1286, 1246, 1167 cm-1.

^{1}H NMR (acetone-d6): 6.26 (1H, d, J=2.5 Hz, H-6), 6.46 (1H, d, J=2.4Hz, H-6^{1}), 6.54 (1H, d, J=2.4 Hz, H-8), 6.69 (1H, s, H-3^{1}),6.76 (1H, s, H-3), 6.85 (2H, d, J=8.8 Hz, H-3$^{1'}$ and H-5$^{1'}$),7.27 (1H, d, J=8.6 Hz, H-5^{1}), 7.68 (2H, d, J=8.8 Hz, H-2^{11}and H6^{11}), 8.06 (1H, q, J=2.4 Hz and 8.6 Hz, H-6^{1}) and 8.16 (1H, d, J=2.4 Hz, H-2^{1}).

^{13}C NMR (acetone-d6): 94.93, 99.67, 99.92, 101.92, 104.47, 105.51, 105.71, 116.85, 117.66, 120.94, 123.44, 123.58, 128.97,129.26, 132.74, 156.26, 158.97, 160.36, 161.93, 162.67, 162.93, 163.49, 164.99, 165.23, 183.19 and 183.58 .HR-ESIMS (positive mode) m/z 561.07800 [M + Na] $^{+}$ (calcd. 561.15668 for $C_{30}H_{18}O_{10}Na$); HR-ESIMS (negative mode)

Compound: 2 TETRA HYDRO AMENTO FLAVANONE
CHEMICAL FORMULA: $C_{30}H_{22}O_{10}$, m/z: 542.12

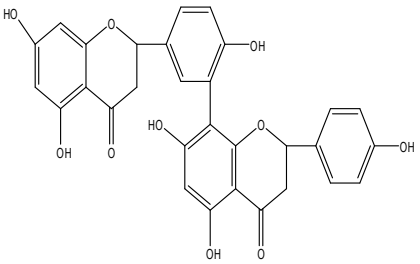
Table1 Elemental concentrations in methanolic soxhlet Leaf , Flowers, Stem bark, Root bark, Nuts extracts and ASH Content of *Semecarpus anacardium L.f* by the ICP-AES technique.

Elements in Semecarpus Anacardium L.f	Wave lengethed in nm	SA FA	SARA	SA LA	SA NA	SA NMe	SA St Et	SAFMe	SALMe	SARMe	SA NH	Permissible limits
B	249.7	2.77	2.0	2.89	5.82	3.05	0.209	1.97	1.33	0.0403	3.62	100
Ca	422.6	202.2	3071.0	376.5	724	5.28	0.0446	24.4	3.59	1.126	14.18	200
Cu	324.7	0.99	2.45	5.35	2.57	0.03	1.0	0.3	0.02	0.0419	0.14	150
K	766.4	653.7	1075	1166.5	1959.5	58.25	0.0239	55.3	3.18	21.66	34.8	200
Mg	280.2	158.9	734.2	237.5	317.5	2.92	0.25	5.98	0.6	2.341	7.4	300
Mn	257.6	1.19	2.4	3.62	3.75	0.02	0.24	0.19	0.01	0.01	0.09	260
Na	588.9	16.68	35.4	29	44.0	13	0.01	5.5	4.14	1.928	8.17	200
Ni	221.6	BD	BD	BD	BD	BD	0.89	BD	BD	0.02	BD	900
Zn	213.8	1.62	1.65	2.86	5.608	0.14	0.022	0.23	0.2	0.1	0.25	1500
Al	309.2	10.7	61.7	17.59	BD	0.76	0.13	1.31	0.58	0.0403	1.23	900
Pb	220.3	0.06	0.03	0.36	0.05	0.05	0.209	0.15	0.07	1.126	0.03	100

Note: BD:Below detection, SA: Semecarpus anacardium L.f; SAFA: Semecarpus anacardium Flower ash; SARA: Semecarpus anacardium Root bark ash; SALA: Semecarpus anacardium Leaf ash; SANA: Semecarpus anacardium Nuts ash; SANMe: Semecarpus anacardium Nuts Methanolic Extract; SASt Et: Semecarpus anacardiumStem bark ethyl acetate extract; SAFMe: : Semecarpus anacardium Flowers Methanolic Extract; SALMe: Semecarpus anacardium Leaf Methanolic Extract; SARMe: Semecarpus anacardium Root bark Methanolic Extract.

Table 2 Elements in *Semecarpus anacardium* L.f. and their Medicinal properties[27]

Elements in *Semecarpus anacardium* L.f	Medicinal properties
Boron	Arthritis
Calcium	Blood pressure for blood clotting& Arthritis
Copper	Regulates the blood pressure, pulse and healing, Arthritis, Wilson's disease and Menke's disease.
Potassium	Deficiency leads to stroke, heart problem, diabetes and hypertension, Potassium is a major dietary mineral that helps balance our body's pH and body fluids. Necessary for normal muscle growth and enzymatic reactions.
Magnesium	Prevents heart disorders and high blood pressure controls Insulin levels in blood. Used in asthma attack situations, It improves the enzyme activity; Deficiency leads to irritability and nervousness. It helps in digestion supports the Immune system. Regulates blood sugar levels.
Manganese	It works with Vitamin K to support blood clotting. Deficiency leads to poor bone formation, affect fertility and ability of blood to clot. It is an antioxidant nutrient, essential for the metabolism of VitaminB1, Vitamin C, Vitamin E, Food supplement to gracing animals.
Sodium	Loss of Na from body leads to dehydration and weakness. Excess of Na may contribute to high blood pressure. It performs the transmission of electrical impulses and the regulation of water content in tissue and blood, used as nutritional supplements.
Nickel	It cause cancer, High concentration of Ni & Zn leads toxic. Low concentration is Important micro nutrient.
Zink	It plays a major role as catalyst, influencing immune system, It maintains various reactions of the body which help to construct and maintain DNA, required for the growth and repair of body tissues.
Aluminum	It can be accumulated in the body from daily intake and at one time was suggested as a potential factor in Alzheimer's disease (Senile dementia), although some studies have disproved this theory. Only a small amount of what we take in with our food is absorbed by our bodies.
Lead	It is a non-essential trace element having functions neither in humans nor plants. They induce toxic effects in humans even at low doses. Lead accumulation results in reduced functioning of kidney, liver and brain cells and later in complete breakdown of the tissues, It is toxic in a cumulative way, teratogenic and carcinogenic.



Compound :2

Its UV absorptions in methanol are at UV (MeOH) $_{max}$ (log): 285(4.52), 225(sh), 330(sh)

IR (KBr) $_{max}$: 3400,1605,1445,1310,1235,1148,1078,820.

^{1}H NMR (acetone-d_6, **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).

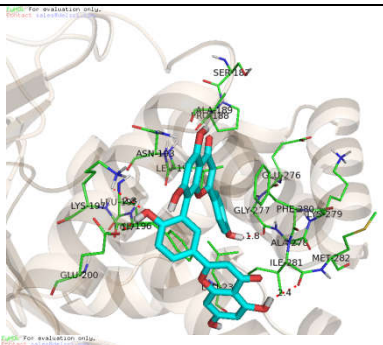
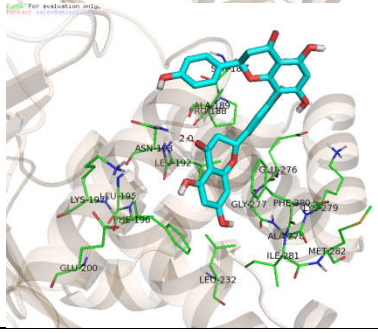
^{13}C NMR (acetone-d_6**400MH z):** δ 43.46, 43.95, 79.86,80.66, 96.25 ,96.94, 97.07, 103.40, 107.01, 116.22, 116.59 , 121.40, 127.96, 128.70, 130.71,131.25, 132.58, 158.63, 162.03, 164.63, 164.99, 165.50 ,165.92 ,168.32, 197.95, 198.42.

residues and probable mode can be used to design of novel Biflavanoid compounds with the improved potency.

In order to explore the binding mode and understanding of key active site residues of extracted natural compounds such as Biflavanoids in Ptp1B, molecular docking study has been performed. The probable binding modes of best docked compounds are shown (in Fig.9 &10). The docking parameters and physicochemical properties of Biflavanoids in PTP1B targets are shown in Table 4&5.

Introduction: Human tyrosine-protein phosphatase non-receptor type 1 (PTB1B, PTP1N) belongs to protein-tyrosine phosphatase family of non-receptor class 1 subfamily. It contains 435 aminoacids. The target is located in endoplasmic reticulum membrane, peripheral membrane protein, cytoplasmic side. It Interacts with EPHA3 (phosphorylated), dephosphorylates EPHA3 and may regulate its trafficking and function. Tyrosine-protein phosphatase which acts as a regulator of endoplasmic reticulum unfolded protein response.

Table 3 Isolated compounds and their 3D Structures

S.NO	COMPOUND NAME	MOLECULAR FORMULA	STRUCTURE	MOLECULAR WEIGHT	3D STRUCTURES
AF	AMENTO FLAVONE	$C_{30}H_{18}O_{10}$		538.46	
THAF	Tetrahydroame-nto flavanone	$C_{30}H_{22}O_{10}$		542.49	

Molecular docking studies on Isolated Biflavanoids

The computational techniques such as molecular docking studies have been applied to support our experimental results. The structures of isolated THAF & AF from *Semecarpus anacardium* L.f. from various parts were characterized. To render support to the experimental results, THAF & AF structures were drawn in Chem Draw and converted into 3D-molecules with all possible tautomers and chiral centers. The minimizations of molecules were carried out using Polak-Ribiere Conjugate Gradient (PRCG) method with maximum of 5000 iterations. The minimized compounds were used for docking against THAF&AF target (PDB ID: 3K84) for understanding their binding modes and key active site residues. The docked structures were compared with congnate biflavanoids reference structure. The identified key active site

It mediates dephosphorylation of EIF2AK3/PERK, inactivating the protein kinase activity of EIF2AK3/PERK. It may play an important role in CKII- and p60c-src-induced signal transduction cascades. It also regulates the EFNA5-EPHA3 signaling pathway which modulates cell reorganization and cell-cell repulsion. It also regulates the hepatocyte growth factor receptor signaling pathway through dephosphorylation of MET. PTP1B29 is a negative regulator of the insulin signaling pathway and is considered a promising potential therapeutic target for treatment of type 2 diabetes. It has also been implicated in the development of breast cancer and has been explored as a potential therapeutic target for cancer therapeutics.

In the present study, the molecular docking study has been conducted with 2 Biflavonoids on PTB1B targets. From the docking study, 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.

METHODOLOGY

All computations and molecular modeling studies were carried out on Schrodinger software.

Protein preparation

The crystal structure of PTB1B (PDB ID: 1T49) have been selected for the docking studies of the PTB1B structures were prepared by adjusting bond orders, tautomers and adding hydrogen atoms using protein preparation wizard of Schrödinger software graphical user interface Maestro v9.3 [30] Further the proteins were minimized by OPLS_2005 force field with converge heavy atoms to RMSD 0.3 Å relative to original protein structure.

Docking studies

The docking study has been carried out on a minimized 2 Biflavanoids with prepared PTB1B proteins using Schrödinger docking program module .The prepared protein structures (3K84, 1T49) were used for grid generation using the default value of protein atom scaling (1.0) within a cubic box centered on the co-crystal ligand. No constraints were imposed and extra precision (XP) docking of ligands was carried out with default value of ligand atom scaling (0.8). The post docking minimization has been carried out and maximum of 10 poses per ligand was saved. The obtained the docked complex structures were analyzed and the compounds were prioritized by using docking score, interactions with active site residues.

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 group hydrogen bond interaction with Ser190 and Glu276. PBF1 showed hydrophobic interactions with Phe196, Ile281, Phe280, Leu192 and Ala189.

AF in the active site of Ptp1B: The two hydroxyl groups in catechol moiety showed hydrogen bond interactions with Lys279. The carbonyl group of chromanone ring showed hydrogen bond interaction with Asn193. PJF-a showed parallel displaced π-π hydrophobic interaction with Phe280 and T-shaped π-π interaction with Phe196 and other hydrophobic interactions include Leu192, Pro188 and Ala189.

Antimicrobial activity of the isolated compounds

The antimicrobial activity of the isolated compounds and their derivatives was determined using well diffusion method [31] 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 bacterial 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°C and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration.

Table 4 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 5 The docking parameters and physicochemical properties of flavanoids in PtpIB target

Flavonoid	gscore	evdw	ecoul	energy	emodel	Mol.wt	logP	PSA
THAF	-6.9	-39.86	-4.08	-43.94	-61.18	542.5	2.76	193.26
AF	-6	-33.03	-13.07	-46.1	-64.47	538.47	2.62	192.59

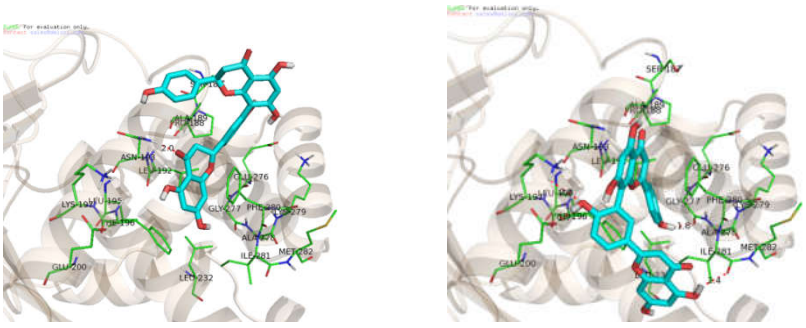


Fig .9&10 3D IMAGES OF THAF&AF

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 (1mM) 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.

herbal drugs. Compounds THAF&AF as well as Methanolic Crude Extracts of whole plant of *Semecarpus anacardium* L.f were the most active against *both gram +ve and gram –ve bacteria*. Compound PFA presented a broad spectrum of activity against both gram positive and gram negative bacteria could therefore be ascertained that the relationship between structures of these compounds and observed biological activity. Phytocomponents in the extract implies the phytopharmaceutical importance of the plant Padmavathi.V and Kesava Rao B*(2013) [32], (2014) [33], [34], [35], [36], (2015) [37], [38], [39], [40], [41], (2016) [42].

Table 6 Anti microbial Activity of Extracts and Pure compounds

COMPOUNDS	*Staphylococcus aureus MTCC 96*	*Klebsiella planticola MTCC 530*	*Bacillus subtilis MTCC 121*	*S.aureus MLS16 MTCC 2940*	*Micrococcus luteus MTCC 2470*	*Escherechia coli MTCC 739*	*Pseudomonas aeruginosa MTCC 2453*	*Candida albicans* MTCC 3017
Root Methanolic extract	+	+	-	-	--	+	+	+
Nuts Methanolic extract	+	+	+	+	--	--	+	+
Flowers Methanolic	+	+	--	--	+	--	+	--
Leaves methanolic	+	+	+	+			+	+
Stem bark methanolc	+	+			+	+	+	+
AF	9.37	18.75	18.75	----	9.37	9.37	18.75	9.37
THAF	**18.75**	**9.37**	**9.37**	9.37	9.37	---	9.37	18.77
Neomycin	18.75	18.75	18.75	18.75	18.75	18.75	18.75	--
Miconazole	--	--	--	--	--	--	--	9.37

The scavenging activity was calculated using the following equation:

$$(\%)\ \text{Free radical scavenging activity} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}} \times 100$$

Table 7 Radical Scavenging Activity of compounds from SA.

Sample Name	% Free radical Scavenging activity
BHA(1Mm)Reference anti oxidant	93.467
AF	70
THAF	72

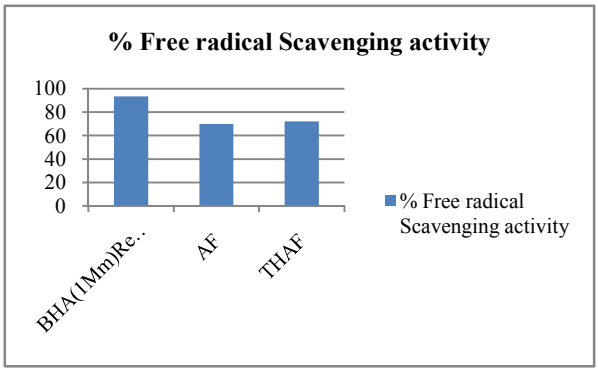


Fig .11 Graphical representation of Radical Scavenging activity of compounds from SA.

CONCLUSION

The monitoring of metals in these plants is of some therapeutic and prophylactic importance The present study is the first report on the essential elements in *Semecarpus anacardium* L.f Leaves Flowers, Root bark, Stem bark, Nuts ,Extracts and Ash contents, essential elements that could enhance the curative process of ill health. The potentially toxic elements were not detected in the present plant extract and Ash. It has been concluded from this study that estimation of heavy metals and trace elements are highly essential to raw drugs used as the medicine, it will help full in quality assurance and safer use of

The computational techniques such as molecular docking studies have been applied to support our experimental results, In order to explore the binding mode and understanding of key active site residues of extracted natural compounds such as THAF & AF, Biflavanoids in Ptp1B, molecular docking study has been performed. The identified key active site residues and required structural features can be used to design the novel compounds for Ptp1B targets in future and toxic effect could support the relevance of functional group substitution in the biological activity. Naturally occurring pure compounds exhibiting good antimicrobial activity which can selectively kill microorganisms without being significantly toxic to host cells can be a useful tool in evaluating the potential toxic effect of compounds in vivo.

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