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## DNA BARCODING AND BIOLOGICAL ASSESSMENT ON A FEW MANGROVE PLANT SPECIES OF VISAKHAPATNAM COAST, ANDHRA PRADESH, INDIA

P. Suman <sup>\*1</sup>, T. Samuel David Raj <sup>2</sup>, P. R. Vani <sup>2</sup> and S. Hara Sreeramulu <sup>1</sup>

Department of Biotechnology <sup>1</sup>, Department of Zoology <sup>2</sup>, Center for Research, Dr. V. S. Krishna Government Autonomous College, Visakhapatnam - 530013, Andhra Pradesh, India.

### Keywords:

DNA Barcoding, Phytochemicals, Mangrove plants, Hepatotoxicity

### Correspondence to Author:

**Polaki Suman**

Research Scholar,  
Project Assistant,  
Department of Biotechnology,  
Center for Research, Dr. V. S. K.  
Government Autonomous College  
Affiliated Andhra University,  
Visakhapatnam - 530013, Andhra  
Pradesh, India.

**E-mail:** [suman.biotech4679@gmail.com](mailto:suman.biotech4679@gmail.com)

**ABSTRACT:** DNA barcoding is currently gaining popularity due to its simplicity and high accuracy as compared to the complexity and subjective biases associated with morphology-based identification of taxa. The standard chloroplast DNA barcode for land plants is normally recommended by the Consortium for the Barcode of Life (CBOL) plant working group that needs to be evaluated for a wide range of plant species. In Visakhapatnam district of Andhra Pradesh state, there are many natural and manmade seasonal wetlands that serve as important gateways for the migratory waterfowl. Hence these wetlands contribute significantly towards balancing of ecosystem. Many researchers had identified plant species of these wetlands by traditional methods but the aspect of identification of plants through barcoding has largely been ignored. We therefore, tested the potential of the rbcL marker for the identification of Mangrove plants belonging to two families of some wetlands of Visakhapatnam. This had been carried out by rbcL gene as the standard plant barcode Maximum likelihood tree analysis was also performed to evaluate the discriminatory power of the rbcL gene. The classical taxonomic classification was then compared with the classification obtained through DNA Barcode tree. Our findings showed that using rbcL gene sequences, majority of the samples, i.e. (100%) were identified at genus and species level. This research work has revealed the potentiality of three mangrove species having hepatoprotective activity which could be useful in the area of life sciences. Further research work is needed to determine the biologically active molecules from *S. prostrata*, *S. microphylla* and *S. portulacastrum*; this would be the basic platform to be executed in various applications of life sciences. The advancements in DNA barcoding studies helps in better understanding of flora or fauna at species level and could pave a way for proving certain phytochemical, anticancer, antioxidant, hepatoprotective activities at molecular level which is more authenticated.

**INTRODUCTION:** DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome. DNA barcode sequences are very short relative to the entire genome and they can be obtained reasonably quickly and cheaply known as “DNA barcodes”, that have been proposed and initiated to facilitate biodiversity studies, identify juveniles, associate sexes, and enhance forensic analyses.

In recent years there has been a drive to speed up the rate at which species on earth are identified and described in response to the diversity of life which is disappearing at an ever increasing rate. There are an estimated 300000 plant species in the world (IUCN, 2012) but relatively few of these can be identified based on traditional plant identification methods <sup>5, 11</sup>.

Accurate classification and identification of this large number of species remains a significant challenge even for specialist taxonomists. The emergence of DNA barcoding has had a positive impact on biodiversity classification and identification. Since it was first put forward and widely applied in animals <sup>11</sup>, DNA barcoding has attracted much attention from taxonomists. DNA barcoding can also be used for a wide range of

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purposes: to support ownership or intellectual property rights<sup>18</sup> to reveal cryptic species<sup>11</sup> in forensics to link biological samples to crime scenes<sup>21</sup> to support food safety and authenticity of labelling by confirming identity or purity<sup>10</sup> and in ecological and environmental genomic studies<sup>19</sup>.

Mangroves are various large, extensive type, salt tolerant trees (halophytes) adapted to live in harsh coastal conditions of trees up to medium height and shrubs that grow in saline coastal sediment habitats in the tropics and subtropics mainly between latitudes 25° N and 25° S. The remaining mangrove forest areas of the world in 2000 was 53,190 square miles (137,760 km<sup>2</sup>) spanning 118 countries and territories. There are about 80 different species of mangrove trees. All of these trees grow in areas with low-oxygen soil, where slow-moving waters allow fine sediments to accumulate. Mangrove forests only grow at tropical and subtropical latitudes near the equator because they cannot withstand freezing temperatures. Many mangrove forests can be recognized by their dense tangle of prop roots that make the trees appear to be standing on stilts above the water. This tangle of roots allows the trees to handle the daily rise and fall of tides, which means that most mangroves get flooded at least twice per day. The roots also slow the movement of tidal waters, causing sediments to settle out of the water and build up the muddy bottom. These forests stabilize the coastline, reducing erosion from storm surges, currents, waves, and tides. The intricate root system of mangroves also makes these forests attractive to fish and other organisms seeking food and shelter from predators.

Numerous mangrove plants are used in folklore medicine. Extracts from mangroves and mangrove dependent species have proven effective against human, animal and plant pathogens, but only limited investigations have been carried out to identify the metabolites responsible for their bioactivities. Skin disorders and sores – including leprosy – may be treated with ashes or bark infusions of certain species of mangrove. Reported to be an astringent, expectorant, hemostat, styptic and tonic, red mangrove is a folk remedy for angina, asthma, backache, boils, constipation,

convulsions, diarrhea, dysentery, dyspepsia, elephantiasis, eye ailments, fever, fungal infections, headaches, hemorrhage, inflammation, jaundice, liver diseases, kidney stones, lesions, malaria, malignancies, rheumatism, snakebites, sores, sore throat, syphilis, toothache, tuberculosis, ulcers and wounds<sup>16</sup>.

The uses of mangroves falls into two categories, firstly the use of the mangrove ecosystem as a whole or its conversion to other uses, and secondly, the use of products from the mangrove ecosystem. Traditionally, people have used mangroves for the benefit of the local community, but increasing populations have led to an increasing non-sustainable abuse of the resources. Mangroves are used in flavouring agents, textiles, mats, paper, housing, baskets, boats and tapa cloth and also used as staple food. The indigenous people of Australia and Sri Lanka use extracts from mangrove plants as valuable sources of dyes.

Among the common mangrove plants the three plant species namely *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastrum* have taken up in the present study. Both *Suaeda species* traditionally have been using as a leafy vegetables and the *Sesuvium portulacastrum* is also having highly nutritive properties in the preparation of pickles in human remedies etc. As such, in the present study on the three above species have taken up due to ambiguity in their identification and assessment roles.

### Objectives:

1. To determine the correct identity by using DNA Barcoding sequence and to establish the phylogenetic relationship among the mangrove plant species studied.
2. To screen the presence of biologically active constituents in the mangrove plant species studied.
3. To determine the hepatoprotective and antioxidant properties of *Suaeda species* ethanolic extract on concanavalin - A induced hepatotoxicity in rats.
4. To determine the Hepatoprotective and antioxidant activities of methanolic extracts of

*Sesuvium portulacastrum* against CCl<sub>4</sub> induced liver injury in rats.

**MATERIALS AND METHODS:** In this study Research Methodology, Results and Discussion are adopted criteria wise on each and every research assay.

#### Research Assay: 1

#### DNA Barcoding and Phylogenetic Analysis of Three Mangrove Plant Species of Visakhapatnam District using *rbcL* primers:

**Tissue Sampling and Storage:** Mangrove plant samples were collected from the coastal regions of Visakhapatnam District for analysis from the natural environmental conditions during the developmental stage and were frozen at - 20°C.

#### Genomic DNA Purification & PCR Protocol:

DNA isolation reagent (Qiagen Genomic DNA purification kit), PCR Master Mix, Agarose gel electrophoresis consumables and Primers are purchased from HELINI Biomolecules, Chennai, India. The DNA extraction kit contains following reagents and their compositions:

#### DNA Purification Buffers:

1. Solution A: contains Tris, EDTA, Guanidine thiocyanate
2. Solution B contains 10% SDS
3. Solution C contains potassium acetate
4. PT Buffer contains isopropanol mix
5. Wash buffer: contains 70% ethanol
6. TE Buffer: contains Tris and EDTA

**2X Master Mix:** It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl<sub>2</sub>, 1 µl of 10mM dNTPs mix and PCR additives.

**Agarose Gel Electrophoresis:** Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide are purchased from HELINI Biomolecules, Chennai.

#### Procedure:

#### Genomic DNA Extraction from Plant Leaves:

1. 10mg of leaf is taken into fresh 1.5ml centrifuge tube.

2. Added 320ml of Solution-A and 80 µl of Solution-B, and grinded with micro pestle, vortexed well till homogenous solution obtained.
3. Incubated in water bath at 65°C for 15 min.
4. Cool to room temperature and Added 130ml of Solution C.
5. Inverted gently for 5 times and incubate for 5 minutes in room temperature.
6. Centrifuge it for 10 min at 10000rpm. It makes precipitated cell debris and denatured proteins to form a pellet.
7. Transferred the supernatant into fresh tube. Supernatant contains DNA.
8. Added equal volume of PT buffer and gently invert 5 times and Incubate for 10 minutes in room temperature. This makes DNA and RNA to precipitate.
9. Centrifuged at 10000rpm for 5 minutes. It makes precipitated DNA to form a pellet.
10. Discard supernatant and saved DNA pellet.
11. Added 500 µl of Wash buffer without disturbing pellet and keep it for 1 min. This solution removes co precipitate salts from the pellet.
12. Centrifuge at 10000rpm for 1 minute and discard the supernatant.
13. Air-dried pellet by inverting it in paper towel for 10 minutes. This makes the wash buffer containing ethanol to evaporate.
14. Suspend the pellet in 100µl of TE Buffer and dissolve completely.
15. Quality of extracted DNA is checked by loading in 1% agarose gel.
16. Use 2 µl of extracted DNA for PCR amplification.

Lane 1: Plant DNA sample- DRVSKCFR1

Lane 2: Plant DNA sample- DRVSKCFR2

Lane 3: Plant DNA sample- DRVSKCFR3

#### PCR Procedure:

[25 µl of Master Mix contains: 10X Taq buffer, 2mM Mgcl<sub>2</sub>, 0.4mM dNTPs mix, and 2U *Proofreading* Taq DNA polymerase].

#### Rbcl Primer:

5´- ATGTCACCACAAACAGAGACTAAAGC - 3´ (forward primer – rbcLa F)

5´- GTAAAATCAAGTCCACCRCG -3´ (reverse primer – rbcLa R)

1. Spin them briefly and keep in ice bucket.
2. Reactions set up as follows;

Components	Quantity
In PCR vial Master mix	25 $\mu$ l
<i>rbcL</i> Primer - forward(10pmoles/ $\mu$ l)	1 $\mu$ l
<i>rbcL</i> Primer- reverse(10pmoles/ $\mu$ l)	1 $\mu$ l
Genomic DNA	2 $\mu$ l
Water, nuclease free	21 $\mu$ l
Total volume	3. 1

3. Mixed gently and spin down briefly.
4. Place into PCR machine and program it as follows;

**Initial Denaturation:** 95°C for 3 min

**Denaturation:** 94°C for 1 min

**Annealing:** 55°C for 1min 35 cycles

**Extension:** 72°C for 1min

**Final extension:** 72° C for 10 min

10 °c for  $\infty$

#### Loading:

1. Prepare 2% agarose gel. [2gm of agarose in 100ml of 1x TAE buffer]
2. Mix 8  $\mu$ l 6X Gel loading dye to each PCR vial and loaded 5  $\mu$ l of PCR sample.
3. Run electrophoresis at 50V till the dye reaches three fourth distances and observe the bands in UV Transilluminator.

#### Agarose Gel Electrophoresis:

1. Prepared 2% agarose. (2gm agarose in 100ml of 1X TAE buffer and melted using micro oven)
2. When the agarose gel temperature was around 60°C, added 5  $\mu$ l of Ethidium bromide.
3. Poured warm agarose solution slowly into the gel platform.
4. Kept the gel set undisturbed till the agarose solidifies.
5. Poured 1X TAE buffer into submarine gel tank.
6. Carefully placed the gel platform into tank. Maintained the tank buffer level 0.5cm above than the gel.
7. PCR Samples are loaded after mixed with gel loading dye along with 10  $\mu$ l 100bp DNA Ladder.
8. Run electrophoresis at 50V till the dye reaches three fourth distance of the gel.

9. Gel viewed in UV Transilluminator and observed the bands pattern.

#### Sample Ladder:

Sample: ~550bp PCR product

QuickRefR DNA Ladder: 100bp, 250bp, 500bp, 750bp and 1000bp.

**PCR and Gene Sequencing:** *rbcL* gene in plants was amplified in a volume of 20  $\mu$ l containing 10  $\mu$ l Taq PCR reaction mix, 10 pmol forward primer (*rbcLa* F – ATGTCACCACAAAC AGAGACTA AAGC), 10 pmol reverse primer (*rbcLa* R – GTA AAATCAAGTCCACCRCG), template DNA (50 mg/ $\mu$ l) and sterile ion-free water (to make up the final desired volume). Amplification was carried out in Thermal cycler (Applied Biosystems VeritiR). Reactions were amplified through 35 cycles with the following conditions<sup>14, 15</sup> Denaturation: 30 seconds at 95°C. Annealing: 40 seconds 55°C, Extension: Two minutes at 72°C. This was followed by a final extension step at 72°C for seven minutes.

Initial denaturation was carried out at 95°C for five minutes. Electrophoresis was carried out at 150V. The gel images were recorded in JPEG or TIF formats using gel documentation system (Biorad, USA). The gels were analyzed by using the software Image lab version 3.0 (Biorad, USA). Purification of *rbcl* gene amplified products were done using GenElute™ PCR Clean-up kit (cat no. NA 1020-1kt) Sequencing was carried out using BigDyeR Terminator v 3.1 Cycle sequencing kit. The BigDye Terminator v3.1 Cycle Sequencing Kit provides the required reagent components for the sequencing reaction in a ready reaction, pre-mixed format. Cycle sequencing was performed in 10  $\mu$ l volume. Purification of cycle sequencing product was done using BigDye XTerminatorR Purification Kit. Kit contains SAM™ Solution and BigDyeR XTerminator™ Solution. Capillary electro phoresis of cycle sequenced products was Performed on 3500 XL platform (Applied biosystems).

**Analysis and Sequence Alignment:** Sequence analysis was performed using sequencing analysis version 5.4 (Applied Biosystems) and Sequence Scanner Software 2; Basic Local Alignment

Search Tool (BLAST) searches were applied to all produced sequences using available online databases (DDBJ/EMBL/GenBank). BLAST was never intended to be used in this manner, but could provide valuable insights into how well we can expect the possibly more appropriate plastid *rbcL* short sequence regions to perform as barcodes<sup>5, 6</sup>. There are very few *rbcL* records on the current BOLD (Barcode of Life Data) identification system (v 2.5)<sup>11</sup> thus, queries might not return an authentic match. Identification at genus level was considered successful when all hits with maximal percent identity scores >95% involved a single genus. Species identification was considered successful only when the highest maximal percent identity included a single species and scored >95%<sup>8</sup>. The *rbcL* sequences were matched with the query sequences and available *rbcL* sequences of the examined plant species; if not available, then genera were retrieved from the DDBJ/EMBL/GenBank databases. Sequence match analysis using BLAST on NCBI. Consensus sequences which showed significant match with the earlier identified data on NCBI were submitted to BOLDSYSTEMS according to the guidelines provided onto BOLD website (<http://www.boldsystems.org/>). For few species where NCBI data was not available were subjected to detailed and thorough morphological analysis and submitted to BOLD.

**Tree-based Analysis:** Phylogenetic analyses were conducted in Genious Basic 5.0.4 software<sup>19</sup> and the phylogenetic trees were inferred with the maximum likelihood method based on the Tamura-Nei model. In phylogenetic analyses, genus identification was considered successful when the unknown sample formed a monophyletic group together with all members of a single genus, with a bootstrap support of >70%. An equal strategy was applied for species-level identification<sup>9</sup>.

## Research Assay: 2

**Preliminary screening of biologically active constituents and total phenolic content of *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastru*:**

**Collection of Plant Materials:** The leaves of *S. prostrata*, *S. microphylla* and *S. portulacastrum*

were collected from Coastal regions of Visakhapatnam mangrove forest (Lat. 8 °34' 59.72" Long. 78 °07'24.96") Andhra Pradesh State. The collected plant specimens were authenticated by the Department of Botany, Andhra University. The specimens were preserved; voucher numbers were given and stored in the Department of Biotechnology, Dr. V. S. K. Government College. The collected samples were uniformly shade dried and it was granulated or powdered by using a blender and sieved in to coarse powder were utilized for the screening of phytochemical constituents qualitatively.

## Plants Voucher Number:

TABLE 1: PLANTS VOUCHER NUMBER

S. no.	Name of the Plant	Voucher Number
1	<i>Suaeda prostrata</i>	DRVSKBT_01012
2	<i>Suaeda microphylla</i>	DRVSKBT_01013
3	<i>Sesuvium portulacastrum</i>	DRVSKBT_01014

## Qualitative Analysis of Phytoconstituents:

**Tests for proteins-xanthoprotein test:** To 1 ml of extract, few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow colour.

**Tests for Resins:** Five milliliter of distilled water was added to the extract and observed for turbidity.

**Tests for Tannins:** About 0.5 gm of the each extract was taken in a boiling tube and boiled with 20 ml distilled water and then filtered added few drops of 0.1% ferric chloride was added mixed well and allowed to stand some time. Observed for brownish green or a blue-black coloration.

**Test for Alkaloid:** 3 ml aqueous extract was stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

**Tests for Saponins:** To 0.5 gm of extracts was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Tests for Cardiac Glycosides:** 1ml of conc. H<sub>2</sub>SO<sub>4</sub> is prepared in a test tube. 5 ml of aqueous extract from each plant sample is mixed with 2 ml of Glacial acetic acid containing 1 drop of FeCl<sub>3</sub>. The above mixture is carefully added to the 1 ml concentrated H<sub>2</sub>SO<sub>4</sub> so that the concentrated H<sub>2</sub>SO<sub>4</sub> is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear, indicating the presence of the cardiac glycosides constituents.

**Test for terpenoids – Salkowski test:** To 0.5 g of the extract, 2 ml of the chloroform was added; Conc. H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of Terpenoids.

**Test for Flavonoids:** A few drops of 1% NH<sub>3</sub> solution is added to the aqueous extract of each plant sample in a test tube. A yellow colouration is observed if flavonoids compounds are present.

**Tests for Acidic Compounds:** To the alcoholic extract sodium bicarbonate solution was added and observed for the production of effervescences.

**Tests for Phenols:** The extracts were taken in water and warmed. To this 2 ml of ferric chloride solution was added and observed for formation of green or blue colour.

**Tests for Glycosides- Keller–Kiliani Test:** About 0.5 ml of alcoholic extracts was taken and subjected to the following test, 1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of conc. Sulphuric acid was added to extract and observed for the formation of reddish brown colour at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

**Total Phenolic Content:** Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g Gallic acid equivalent using the

standard curve equation:

$$y = 0.006x + 0.038, R^2 = 0.999$$

Where y is absorbance at 760 nm and x is total phenolic content in the extracts of *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastrum* expressed in mg/gm.

### Research Assay: 3

#### Hepatoprotective and antioxidant properties of *Suaeda species* ethanolic extract on concanavalin-A induced hepatotoxicity in rats:

As the two species of *Suaeda* showing similar phytochemical constituents in the present study with the results of Preliminary phytochemical screening of the ethanolic extracts of the leaves of *Suaeda microphylla* have taken up for the present hepatoprotective study.

**Extraction:** The fresh elder leaves of *Suaeda microphylla* were collected from Visakhapatnam mangrove forest (Lat. 17° 42' 40.65" N and Lon. 83° 15' 25.99" E), Visakhapatnam District, Andhra Pradesh State, India and their identity was confirmed by following the standard monograph<sup>4</sup>. The specimen sample was also authenticated using DNA Barcoding analysis at Department of Genetic Engineering, SRM University, and conformed by Prof. M. Parani, Prof. S. Hara Sreeramulu, Department of Botany, Plant taxonomist, Centre for Research Studies, Dr. V. S. Krishna Government Autonomous College, Affiliated to Andhra University, Visakhapatnam, Andhra Pradesh, India.

The leaves were washed thrice with distilled water to remove the contaminants and air dried in shade. Coarsely powdered sample (500 g) was defatted with petroleum ether (60 - 80°C) and then extracted with 1L of 95% (V/V) ethanol and water mixture by percolation method. The extract was concentrated under vacuum to the solvent free residues. The percentage yield of extract was 52.64% (w/w). Analysis of phytochemical constituents was carried out by standard qualitative tests.

**In vitro Antioxidant Assay:** Determination of DPPH assay, hydroxyl radical scavenging assay,

nitric oxide radical scavenging assay and superoxide radical scavenging assay were performed with various concentrations (1.9 to 500 µg/ml) of *S. microphylla* leaf extract and various concentrations of (1.9 to 500 µg/ml) vitamin C (positive control). The IC<sub>50</sub> values were calculated using linear regression with Statplus pro 2009 software package.

**Treatments:** Male Wistar albino rats (150-200 g) were maintained under standard conditions (23 ± 2°C, relative humidity 55 ± 10% and 12:12 h LD cycle) and allowed free accesses to food (Cell and Molecular biology research Center, AHERF) and water. Experimental protocols were approved by College of Pharmacy, Institutional Animal Ethics Committee, (AHER/CMBRC/212/PO/08/15). For the determination of median lethal dose (LD<sub>50</sub>) animals were kept fasting for overnight providing only water, after which the extracts were administered orally at different doses of 250-5000 mg/kg and all the rats were observed for the physical signs of toxicity for 14 days. If the mortality was observed in 6 out of 9 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 3 animals, then the same dose was repeated again to confirm the toxic dose. One tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity through following treatments.

The animals were divided into 6 groups consisting of 6 per group.

**Group I** (vehicle control) animals were treated with distilled water (5 ml/kg body wt) for 9 days and kept as control group.

**Group II** (hepatotoxin group) animals were treated with distilled water (5 ml/kg body wt) for 9 days + single dose of (12 mg/kg body wt, iv) concanavalin-A on 9<sup>th</sup> day with liquid paraffin (1:1) and hepatotoxin group.

**Group III** treated with silymarin at the dose of 100 mg/kg body wt, was administered through oral gavage for 9 days + single dose of (12 mg/kg body wt, iv) concanavalin-A on 9<sup>th</sup> day with liquid paraffin (1:1) and kept as positive control group.

Animals of group IV, V and VI were treated with ethanolic extracts of 75, 150 and 300 mg/kg body wt. were administered through oral gavage for 9 days + single dose of (12 mg/kg body wt., iv) concanavalin-A on 9<sup>th</sup> day with liquid paraffin (1:1) and kept as treatment groups.

**Analysis of Clinical Parameters:** On the 10<sup>th</sup> day all the animals were anesthetized with ether. Blood samples were collected from jugular vein. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600 × g for 15 min and analyzed for AST, ALT, ALP and bilirubin. Blood standard commercial kit (CML-Biotech PVT. Ltd, India) were used to measure the level of AST, ALT, ALP and Bilirubin. All the determinations were carried out by an auto analyzer of Merck make (300TX, E-Merck-Micro Labs, Mumbai). The hepatoprotective activity (% H), was calculated as follows:

$$1 - \frac{(T - V)}{(C - V) \times 100}$$

Where T is mean value of drug and concanavalin-A, C mean value of concanavalin-A alone and V is the mean value of normal control animals. Values are expressed as mean ± SD which for biochemical parameters statistically using one way ANOVA for comparison with control group and concanavalin-A treated group. *P* < 0.05 was considered as significant.

**Histopathological Studies:** for the histopathological study, the liver of 6 animals from each group were immediately removed after autopsy and the tissues were fixed in bouin's solution for 12 h, and then embedded in paraffin using conventional methods<sup>7</sup> and cut in to 5 µm thick sections and stained using haematoxylin-eosin dye and finally mounted in diphenyl xylene. The sections were observed under microscope for histopathological changes. Histological damage was expressed using the following score system: - absent; ⊥ few; + mild; ++ moderate; +++ severe; ++++ extremely severe (the liver of 6 animals in every group were examined with 10 different microscopic fields).

**Research Assay: 4****Hepatoprotective and Antioxidant Activity of Methanolic Extracts of *S. portulacastrum* (L.) against CCl<sub>4</sub> –Induced Liver Injury in Rats:**

Plant Material *Sesuvium portulacastrum* was collected from were collected from Visakhapatnam mangrove forest (Lat. 17° 42 ' 40.65" N and Lon. 83° 15 ' 25. 99 " E), Visakhapatnam District, Andhra Pradesh State, India and their identity was confirmed by following the standard monograph<sup>4</sup>. The specimen sample was also authenticated using DNA Barcoding analysis at Department of Genetic Engineering, SRM University, and conformed by Prof. M. Parani, Prof. S. Hara Sreeramulu, Department of Botany, Plant taxonomist, Centre for Research Studies, Dr. V. S. Krishna Government Autonomous College, Affiliated to Andhra University, Visakhapatnam, Andhra Pradesh, India.

**Preparations of Plant Extract for Phytochemical Screening and Hepatoprotective Studies:**

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using methanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extract were used for hepatoprotective studies. Normal healthy male Wistar albino rats (180 - 200g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25 ± 2 °C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water “*ad libitum*”.

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose

administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose, if mortality was not observed the procedure was repeated for higher doses such as 150 and 300 mg/kg body weight.

**Experimental Design:** In the investigation a total of 30 rats (25 CCl<sub>4</sub> hepatic toxicity induced rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

**Group I:** Rats received normal saline was served as a normal control.

**Group II:** CCl<sub>4</sub> hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl<sub>4</sub> for 14 days.

**Group III:** Liver injured rats received methanol extract of whole plant of *Sesuvium portulacastrum* at the dose of 150mg/kg body weight for 14 days.

**Group IV:** Liver injured rats received methanol extract of whole plant of *Sesuvium portulacastrum* at the dose of 300mg/kg body weight for 14 days.

**Group V:** Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

**Biochemical Analysis:** The animals were sacrificed at the end of experimental period of 14 days decapitation. Blood was collected, sera separated by centrifugation at 3000kg for 10 minutes. Serum protein and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was spectrophotometrically by using the method of Reitman<sup>166</sup>. Serum alkaline phosphatase (ALP) was measured by the method of king and Armstrong<sup>13</sup>. Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw<sup>1</sup>. The unconjugated bilirubin concentrations were calculated as the difference between total and



conjugated bilirubin concentrations. Gamma-glutamyl transpeptidase (GGTP) was estimated by the method of Szasz<sup>19</sup>. Liver homogenates (10% w/v) were prepared in ice cold 10mM tris buffer (pH 7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa. Enzymatic antioxidants, superoxide dismutase (SOD), Catalase and non enzymatic antioxidant glutathione peroxidase (GPx) glutathione reductase (GRD) and reduced glutathione (GSH) were also assayed in liver homogenates.

**Statistical Analysis:** The data were expressed as the mean  $\pm$  S.E.M. The difference among the means has been analyzed by one-way ANOVA.  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$  were considered as statistical significance using SPSS Software.

## RESULTS AND DISCUSSION:

### Research Assay: 1

#### DNA Barcoding and Phylogenetic Analysis of three Mangrove Plant Species of Visakhapatnam District using *rbcL* Primers:

Various protocols for DNA extraction have been successfully applied to many plant species<sup>21</sup>. These protocols have been further modified to provide DNA suitable for several kinds of analyses<sup>20</sup>. Thus the protocol derived for both

genomic DNA isolation and PCR is efficient, inexpensive, simple, and rapid and yields pure DNA amplifiable by PCR. DNA barcoding analysis is essentially a technique to generate a molecular “fingerprint” for individual species. This technique depends on using random primers (short sequences of DNA) to generate the barcode pattern and analysis of the barcodes can be used to determine the genetic variation inherent in the different chromatogram individuals and the population.

In this study we have demonstrated the utility of *rbcL* as marker for DNA barcoding of three mangrove plant samples like DRVSKCFR 1, DRVSKCFR 2 and DRVSKCFR 3 and we are able to amplify and sequenced the isolated genome of three mangrove samples with *rbcL* as a marker gene, and we get barcode sequence for three mangrove plant species. With the help of BLAST tool and each sequence shows 100% similarity with mangrove plant species *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastrum* respectively (Table 2). The *rbcL* sequences were obtained for 3 samples in addition to the 16 sequences from GenBank. The complete data Set spanned single order Caryophyllales but two different families like *Suaeda sps* in the Amaranthaceae and for *Sesuvium sps* in the Aizoaceae.

**TABLE 2: DATABASE SEARCH MATCH FOR SIMILARITIES AND PHYLOGENETIC RELATIONSHIP USING *rbcL* GENE SEQUENCES AND GENBANK SUBMITTED DNA BARCODE WITH THEIR ACCESSION NUMBER**

S. no.	Morphological Identification	BLAST Search Match	BLAST Similarity (%)	Phylogenetic Affinity	GenBank accession no
1	<i>Suaeda prostrata</i>	<i>Suaeda prostrata</i>	100	<i>Suaeda sps</i>	HM131801
2	<i>Suaeda microphylla</i>	<i>Suaeda microphylla</i>	100	<i>Suaeda sps</i>	HM131798
3	<i>Sesuvium portulacastrum</i>	<i>Sesuvium portulacastrum</i>	100	<i>Sesuvium sps</i>	KJ773883

**Interspecific Variation:** Phylogenetic methods were applied to conduct the study of barcoding species using each barcode locus taken alone and in combinations to evaluate species recovery. At the interspecific level within genera, *Suaeda* was the one genus in the Amaranthaceae available here with more than one species, i.e., *S. prostrata*, *S. microphylla*, *S. linearis*, *S. crassiflora*, *S. maritima*, *S. heteophylla*, *S. calceoliformis*, *S. occidentalis*, *S. vera*, *S. linifolia*, *S. vermiculata*, *S. aegyptiaca*, *S. fruticosa*, and *S. taxifolia* likewise 14 different species were there. The interspecific

variation among these species shows that sample DRVSKCFR1, *Suaeda prostrata* having a close relation with *S. crassiflora* and *S. maritima* which differed by 487 bp.

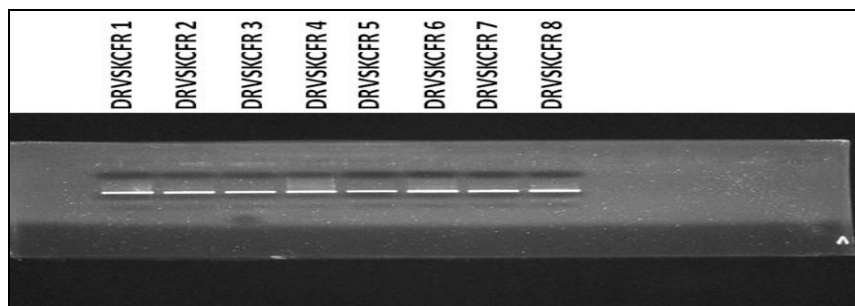
Among these two *S. maritima* shows more interspecific relation than *S. crassiflora*; second sample DRVSKCFR2, *Suaeda microphylla* also having a close relation with other species like *S. fruticosa* and *S. taxifolia* which differed by 85 bp. Among these two *S. fruticosa* shows more interspecific relation than *S. taxifolia*. But *Suaeda*

*prostrata* and *Suaeda microphylla* even though same genus different in their species both of them in different clades and they are distant from each other which are differed by 487 bp. The other plant sample DRVSKCFR3, *Sesuvium portulacastrum* which comes under other new clade but its showing interspecific relation with *Suaeda linearis* and *Trianthea portulacastrum* which differed by 212 and 489 bp. Both *Suaeda linearis* and *Trianthea portulacastrum* belongs to different genus but shows interspecific relation than *Sesuvium verrucosum*. The results indicate that it is possible to discriminate between Mangrove plant species of using *rbcL* sequence data and thus this marker has the potential to be a powerful tool for DNA barcoding. The data set presented here clearly indicates the potential of using *rbcL* for DNA barcoding. The results have highlighted several exciting areas for further research and contribute to ongoing studies, including the need for more critical morphological work.

The clades formed in the trees were mostly mixtures of several species. Therefore, establishing a local barcode database will be valuable for a broad range of potential ecological applications, including the building of community phylogenies<sup>14</sup>. Morphological identification is inapplicable when studying population biology. In such cases, barcoding is an efficient and valuable technique. Some taxonomists have started using the barcoding approach to identify specific unknown plant samples for practical purposes.

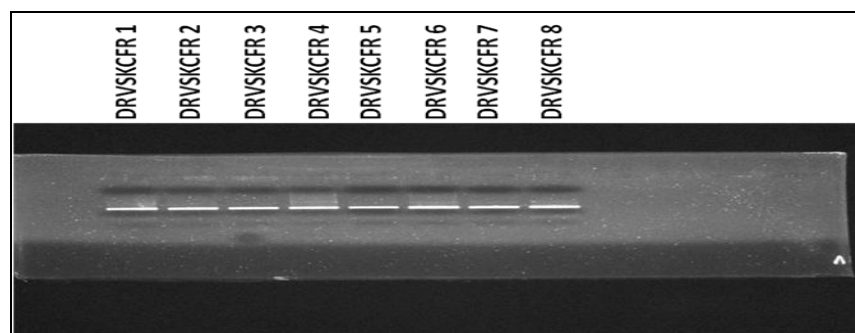
Ongoing developments of new primers and improvements in sequencing techniques have facilitated the data-emergence process of plant barcoding. Since genetic information does not cover the morphology, chemical profile, quality control should always try to consider different techniques. It is advisable to establish a plant digital library of all *rbcL* sequences for international use to allow rapid detection.

#### Isolated DNA Sample:



**FIG. 1: GENOMIC DNA ISOLATED FROM THE MANGROVE PLANT LEAVES RESOLVED UNDER 2% AGAROSE GEL.** Lane 1 represents the isolated genomic DNA from the sample DRVSKCFR 1(*Suaeda prostrata*), Lane 2 represent the isolated genomic DNA from the sample DRVSKCFR 2(*Suaeda microphylla*) and Lane 3 represents the isolated genomic DNA from the sample DRVSKCFR 3 (*Sesuvium portulacastrum*)

#### Quantification DNA (PCR):



**FIG. 2: AGAROSE GEL (1.5%) SHOWING THE PCR QUANTIFICATION OF GENOMIC DNA MANGROVE PLANT SAMPLES.** (Lane 1: DRVSKCFR1 (*Suaeda prostrata*), Lane 2: DRVAKCFR2 (*Suaeda microphylla*) and Lane 3: DRVSKCFR3 (*Sesuvium portulacastrum*).

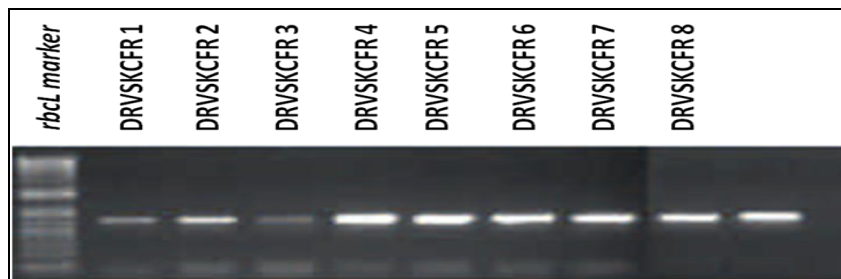
**Sequence PCR Product:**

FIG. 3: AGAROSE GEL (1.5%) SHOWING THE PCR AMPLIFICATION OF REPRESENTATIVE DNA SAMPLES ALONG WITH 2Kb DNA LADDER AT Lane 1; Lane 2, Lane 3 and Lane 4 mangroves with rbcL marker gene

**Classification, Taxonomical Description and DNA Barcoding Sequences:****Sample 1: DRVSKCFR 1: *Suaeda prostrata***

FIG. 4: *SUAEDA PROSTRATA* (PALL)

**Classification:**

Kingdom : Plantae  
 Class : Dicotyledons  
 Order : Caryophyllales  
 Family : Amaranthaceae  
 Genus : *Suaeda*  
 Species : *prostrata*

**Taxonomical Description:** Erect, ascending herbs or under shrubs, stems reddish-purple, leaves linear, crowded, flowers in clusters, whitish green, utricles ovoid.

**Genbank Accession no:** HM131801, *Suaeda prostrata* voucher, ribulose - 1, 5 biphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast; 856 bp linear DNA.

TTGACTTATTATACTCCTGAGTATGAAACC  
 CAAGATACTGATATCTTGGCAGCATTTCGA  
 GTAAGTCCTCAACCAGGAGTTCCACCCGA  
 AGAAGCAGGGGCTGCAGTAGCTGCCGAAT  
 CTTCTACTGGTACATGGACA ACTGTATGGA

CCGACGGACTTACTAGTCTTGATCGTTACA  
 AAGGGCGATGCTACCACATCGAGCCTGTT  
 GCTGGAGAAGAAAATCAATATATTTGTTA  
 TGTAGCGTATCCCTTAGACCTTTTTGAAGA  
 AGGTTCTGTTACTAACATGTTTACTTCCAT  
 TGTGGGTAACGTATTTGGGTTCAAAGCTCT  
 GCGTGCTCTACGTTTGGAGGATTTGCGAAT  
 CCCTGTTGCTTATATAAAA ACTTTCCAAGG  
 ACCACCTCACGGTATCCAGGTTGAAAGAG  
 ATAAATTGAACAAGTACGGCCGTCCCCTA  
 TTGGGATGCACTATTA AACCTAAATTGGG  
 GTTATCTGCTAAAACTATGGTTCGAGCAG  
 TTTATGAATGTCTTCGTGGTGGACTTGATT  
 TTAATAAAGATGATGAAAACGTGAATTCA  
 CAACCGTTTATGCGTTGGAGAGATCGTTTC  
 CTATTTTGTGCCGAAGCTCTTTATAAAGCA  
 CAGGCCGAAACAGGTGAAATCAAAGGGC  
 ATTACTTGAATGCTACTGCGGGTACATGCG  
 AAGACATGATGAAAAGGGCTGTATTTGCC  
 AGAGAATTGGGAGTTCTTATTGTAATGCA  
 TGACTACTTAACAGGTGGATTCACTGCAA  
 ATACTACCTTGGCTCATTATTGCCGAGATA  
 ATGTCTACTTCTTCACATCCACCGTGCAAT  
 GCATGCAGTTATTGATAGACAGAAGAATC  
 A

**Sample 2: DRVSKCFR 2: *Suaeda microphylla***

FIG. 5: *SUAEDA MICROPHYLLA* (PALL) KUNTZE  
**Classification:**

Kingdom : Plantae  
 Class : Dicotyledons  
 Order : Caryophyllales  
 Family : Amaranthaceae  
 Genus : *Suaeda*  
 Species : *microphylla*

**Taxonomical Description:** Ascending herb or under shrub, Stems much branched, woody and red in colour, Leaves long, linear, Flowers ovoid, Fruit ovoid.

**Genbank Accession No:** HM131798 *Suaeda microphylla*, ribulose-1,5-bisphosphate Carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast; 1343 bp Linear DNA.

TTGACTTATTATACTCCTGAGTATGAAACC  
 CAAGATACTGATATCTTGGCAGCATTTCGA  
 GTAACCTCAACCTGGAGTTCCACCTGAA  
 GAAGCAGGGGCTCAGTAGCTGCCAATCT  
 TCTACTGGTACATGGACAACCTGTATGGAC  
 CGACGGACTTACTAGTCTTGATCGTTACAA  
 AGGACGATGCTACCACATCGAGCCTGTTG  
 CTGGAGAAGAAAATCAATATATTTGTTAT  
 GTAGCGTATCCCTTAGACCTTTTTGAAGAA  
 GGTTCTGTTACTAACATGTTTACTTCCATT  
 GTGGGTAACGTATTCGGGTTCAAAGCTCT  
 ACGTGCTCTACGTTTGAAGATTTGCGAAT  
 CCCTGTTGCTTATGTAAAACTTTCCAAGG  
 ACCGCCTCACGGTATCCAGGTTGAGAGAG  
 ATAAATTAACAAGTATGGTCGTCCTTAT  
 TGGGATGCACTATTAACCTAAATTGGGG  
 TTATCTGCTAAAACTACGGTCGAGCAGTT  
 TATGAATGTCTTCGAGGTGGACTTGATTTT  
 ACCAAAGATGATGAAAACGTGAATTCACA  
 ACCGTTTATGCGTTGGAGAGACCGTTTCCT  
 ATTTTGTGCCGAAGCTCTTTATAAGCACAG  
 GCCGAAACAGGTGAAATCAAAGGACATTA  
 CTTGAATGCTACTGCGGGTACATGCGAAG  
 ACATGATGAAAAGGGCTGATTTGCCAGA  
 GAATTGGGAGTTCCTATCGTAATGCATGA  
 CTACTTAACAGGTGGATTCCTGCAAATA  
 CTACCTTGGCTCATTATTGCCGAGATAATG  
 GTCTACTTCTTCACATCCACCGTGCAATGC  
 ACGCAGTTATTGATAGACAGAAGAATCAT  
 GGTATGCACTTCCGTGTACTAGCTAAAGC  
 GTTACGTCTGTCTGGTGGAGATCATATTCA  
 TGCTGGTACCGTAGTAGGGAAGCTTGAAG  
 GGGAAAGAGATATTACTTTGGCTTTGTTGA

TTTACTACGTGATGATTATACTGAAAAAG  
 ACCGAAGCCGCGGTATCTATTTCACTCAAT  
 CTTGGGTTTCTACCCCGGGTGTCTGCCTG  
 TTGCTTCGGGAGGTATTCACGTTTGGCATA  
 TGCCTGCTTAAACCGAGATCTTTGGAGATG  
 ATTCTGTACTACAGTTTGGTGGAGGAACCC  
 TAGGACACCCTTGGGGAAATGCACCAGGT  
 GCTGTAGCGAATCGAGTAGCTCTAGAAGC  
 ATGTGTACAAGCTCGTAATGAGGGGCGTG  
 ATCTTGCTCGCGAAGGTAATACAATTATTC  
 GCGAGGCTAGCAAATGGAGTCTCTGAATTA  
 GCTGCTGCTTGTGAGATATGGAAGGAAAT  
 CAAATTTGAATT

**Sample 3: DRVSKCFR 3: *S. portulacastrum***



**FIG. 6: *SESUVIUM PORTULACASTRUM* (LINN.)**

**Classification:**

Kingdom : Plantae  
 Class : Dicotyledons  
 Order : Caryophyllales  
 Family : Aizoaceae  
 Genus : *Sesuvium*  
 Species : *portulacastrum*

**Taxonomical Description:** Succulent herb, Stems creeping, Leaves narrowed towards base, lanceolate, Flowers purple, Capsules very small and Seeds are black.

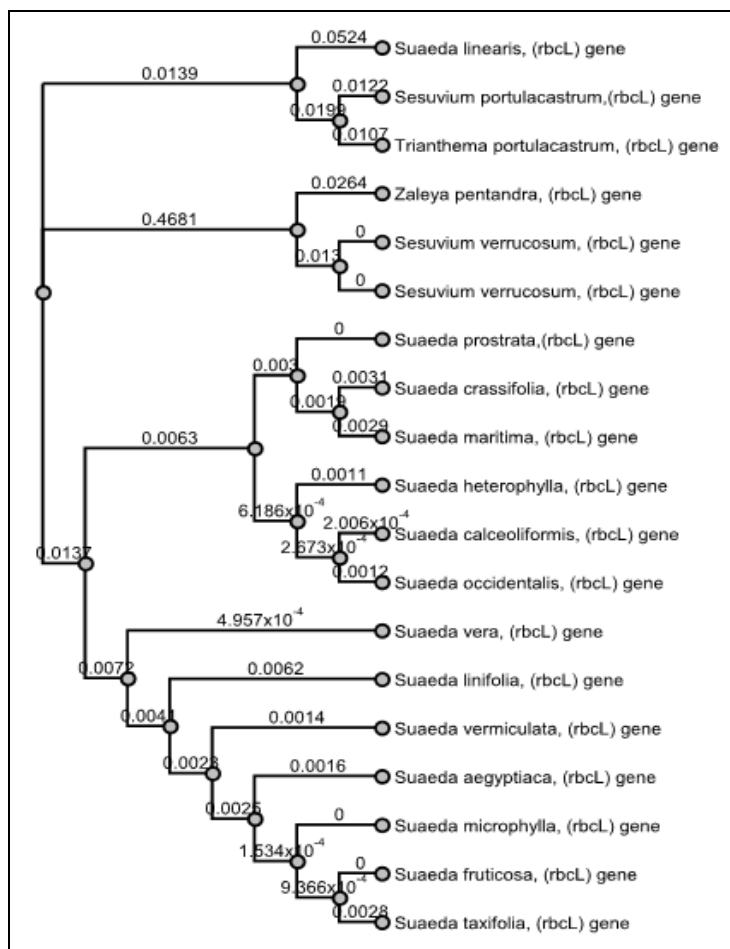
**Genbank Accession no:** KJ773883, *Sesuvium portulacastrum* ribulose - 1, 5 bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast; 1216 bp linear DNA.

AGCTGCCGAATCTTCTACTGGTACATGGAC  
 AACTGTATGGACCGATGGACTTACCAGTC  
 TTGATCGTTACAAAGGACGATGCTACCAC  
 ATTGATCCCGTTCCTGGAGAAGACAATCA  
 ATATATTTGTTATGTAGCTTACCCTTAGA

CCTTTTTGAGGAAGGGTCTGTTACTAACAT  
 GTTACTTCCATTGTGGGTAATGTATTTGG  
 GTTCAAAGCCCTGCGTGCTCTACGTTTGA  
 AGATTTACGAATCCCTATTTCTTATATCAA  
 AACTTTCCAAGGCCCGCCTCACGGTATCCA  
 GGTTGAAAGAGATAAATTGAACAAGTACG  
 GCCGTCCCCTATTGGGATGCACTATTAAC  
 CGAAATTGGGGTTATCCGCTAAAAACTAC  
 GGTCGAGCAGTTTATGAATGTCTTCGCGGT  
 GGACTTGATTTTACCAAAGATGATGAAAA  
 CGTGAACTCCCAACCATTTATGCGTTGGAG  
 AGACCGTTTCTTATTTTGTGCCGAAGCACT  
 TTATAAAGCACAGGCCGAAACAGGTGAAA  
 TCAAAGGGCATTACTTGAATGCTACCGCG  
 GGTACATGCGAAGAAATGATAAAAAGGGC  
 TGTATTTGCCAGAGAATTGGGAGCTCCGA  
 TCGTAATGCATGACTACTTAACAGGGGGA  
 TTCACTGCAAATACTAGCTTGGCTCATTAT  
 TGCCGAGACAATGGTCTACTTCTTCACATC

CACCGTGCAATGCACGCAGTTATTGATAG  
 ACAGAAGAATCATGGTATGCACTTCCGTG  
 TACTAGCTAAAGCGTTACGTCTGTCTGGTG  
 GAGATCATATCCACGCGGGTACCGTAGTA  
 GGTAACCTTGAAGGGGAAAGAGATATCAC  
 TTTAGGCTTTGTTGATTTATTACGTGATGA  
 TCATACTGAAATAGATCCAAATCGT  
 GGTATTTATTTCACTCAATCTTGGGGTTCC  
 ACACCAGGTGTTCTGCCCGTTGCTTCCGGA  
 GGTATTCATGTTTGGCACATGCCTGCTCTA  
 ACTGAAATTTTTGGTGATGATTCCGTACTA  
 CAGTTCGGTGGAGGAACTTTAGGACACCC  
 TTGGGGAAATGCACCGGGTGCTGTAGCGA  
 ATCGAGTAGCTCTAGAAGCATGTGTACAA  
 GCTCGTAATGAGGGACGTGATCTTGCTAC  
 CGAGGGTAATACAATTATTTCGCGACGCTG  
 CCAAATGGAGTCCTGAACTAGCGGCTGCT  
 TGTGAAGTATGG

**Phylogenetic Tree:**



**FIG. 7: PHYLOGENETIC AFFINITIES OF rbcL GENE SEQUENCES OF THE PLANT SAMPLES. THE EVOLUTIONARY HISTORY WAS INFERRED BY USING THE MAXIMUM LIKELIHOOD METHOD BASED ON THE TAMURA-NEI MODEL (1993) USING GENEIOUS BASIC 5.0.4 SOFTWARE**

**Research Assay: 2****Preliminary Screening of biologically active constituents and total phenolic content of *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastrum*:**

**Qualitative Analysis:** Primary metabolites are directly involved in normal growth, development and reproduction. Secondary metabolites are not directly involved in those processes, but usually have an important ecological function. The phytoconstituents of *Suaeda prostrata*, *Suaeda microphylla* and *Sesuvium portulacastrum* were qualitatively analyzed and the results were depicted in the **Table 3**. It indicates that *S. prostrata*, *S. microphylla* and *S. portulacastrum* leaves possess some important primary and secondary metabolites viz., Protein, Resin, Tannin, Glycosides, Cardiac glycosides, Terpenoids, Phenol, Flavonoid, Acidic compounds. Alkaloids and Saponins are absent in both *Suaeda prostrata* and *Suaeda microphylla* where as present in case of *Sesuvium portulacastrum*. Primary and secondary metabolites are very much important for the regular mechanism/survival of the species and also it can be used as therapeutic agents.

Flavanoids are associated with antioxidant, fever-reducing (antipyretic), pain-relieving (analgesic) and spasm-inhibiting (spasmolytic) activities. The decoction of the leaves is used in the treatment of fevers and the flower has soothing properties which are used to relieve menstrual cramps and relax spasms and general cramping. Tannins are used to prevent urinary tract infection and intestinal disorders such as dysentery and diarrhoea. Cardiac glycosides were found to be

present in *C. asiatica* a compound that has been shown to aid in treatment for congestive heart failure and cardiac arrhythmia. This is another reason why this plant is widely used in traditional medicine. Cardiac glycosides work by inhibiting the Na /K pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of  $Ca^{2+}$  ions available for contraction of the heart muscle, improves cardiac output and reduces distension of the heart. *E. officinalis* is also believed to be an aphrodisiac and is considered to be one of the strongest rejuvenate herbs in Ayurveda medicine. It is the primary ingredient used in one of the renowned Ayurveda herbal formula, called Chyavanaprasha which has great respect as a sexual vitality tonic <sup>2</sup>.

This may be contributed by the presence of phenolic compounds, which acts as stimulating agent <sup>3</sup>. *E. officinalis* is also used to detoxify blood from chemicals and harmful toxic due to the presence of phenols, so that it acts as a detoxifying agent. Decoctions of the leaves and seeds of *E. officinalis* are used in the treatment of diabetes and this may be due to the presence of terpenoids in *E. officinalis*. Jayanta Kumar <sup>12</sup> reported that the potential for developing antimicrobial agents from mangrove species due to the presence of phytoconstituents. Similarly *S. prostrata*, *S. microphylla* and *S. portulacastrum* contains the group of biologically active molecules viz., Tannin, Glycosides, Cardiac glycosides, Terpenoids, Phenol and Flavonoid and its medicinal properties were discussed.

**TABLE 3: QUALITATIVE ANALYSIS OF PHYTOCONSTITUENTS IN *SUAEDA PROSTRATA*, *SUAEDA MICROPHYLLA* AND *SESUVIUM PORTULACASTRUM***

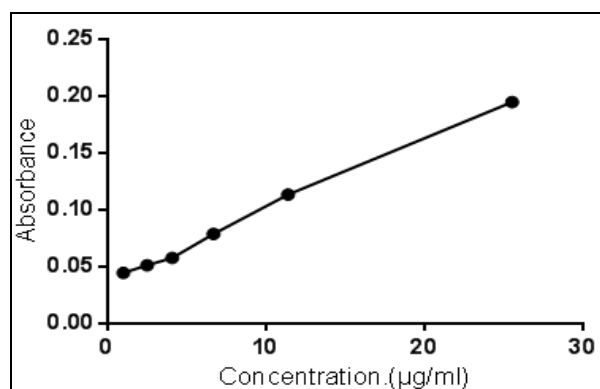
S. no.	Phytoconstituents	<i>S. prostrata</i>	<i>S. microphylla</i>	<i>S. portulacastrum</i>
1	Protein	+	+	+
2	Resins	+	+	+
3	Tannins	+	+	+
4	Alkaloids	-	-	+
5	Cardiac glycosides	+	+	+
6	Terpenoids	+	+	+
7	Flavanoids	+	+	+
8	Saponins	-	-	+
9	Phenols	+	+	+
10	Acidic Compounds	+	+	+
11	Glycosides	+	+	+

(+) Present (-) Absent

**Table 4** shows the variation of mean absorbance with concentration of Gallic acid. **Table 5** shows the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of Gallic acid equivalent. The total phenol varied from 89.482 to 93.332 mg/g in the extracts.

**TABLE 4: ABSORBANCE OF STANDARD COMPOUND (GALLIC ACID)**

Concentration (µg/ml)	Absorbance (Mean) $\lambda_{\max} = 760 \text{ nm}$
1.0	0.0445
2.5	0.0512
4.1	0.0575
6.7	0.0787
11.4	0.1133
25.5	0.1945



**FIG. 8: STANDARD CURVE OF GALLIC ACID**

$$y = 0.006x + 0.038 R$$

**TABLE 5: TOTAL PHENOLIC CONTENT IN SUAEDA PROSTRATA, SUAEDA MICROPHYLLA AND SESUVIUM PORTULACASTRUM**

Seaweed extracts	Total phenol(mg/g)
<i>Suaeda prostrata</i>	92.354
<i>Suaeda microphylla</i>	93.332
<i>Sesuvium portulacastrum</i>	89.482

### Research Assay: 3

#### Hepatoprotective and Antioxidant Properties of *Suaeda species* ethanolic extract on concanavalin-A induced hepatotoxicity in rats:

In Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study with the results of Preliminary phytochemical screening of the ethanolic extracts of the leaves of *Suaeda prostrata* and *Suaeda microphylla* showed the presence of reducing sugars, steroids, and triterpenes. All the *Suaeda species* are having same phytochemical constituents so one such

extract of *Suaeda microphylla* was taken for the hepatoprotective study. In the toxicity study, no mortality occurred throughout the experiment with the doses of plant extract and silymarin treated group. The ethanolic extract did not show any mortality up to the level of 3000 mg/kg and were considered as safe. Single dose of concanavalin-A is sufficient for the development of liver lesions. Concanavalin-A induced hepatitis is both T-cell and macrophage dependent. The precise mechanism(s) by which T-cells and macrophages exert their hepatogenic potential is not known. Because a massive release of macrophage and T-cell derived cytokines IL-2, IL-1, IL-6, tumor necrosis factor-alpha (TNF- $\alpha$ ), interferon-gamma (IFN- $\gamma$ ) and granulocyte macrophage colony stimulating factor (GM-CSF) occurs with different kinetics in response to concanavalin-A, a role has been envisaged for these cytokines in the development of the hepatic lesions.

It is evident that, the concanavalin-A produced liver inflammation due to the severe hepatic necrosis. It is known that an increasing the enzymatic activity of ALP and AST in the serum directly reflects the major permeability of cell membrane. An increase in AST and ALT, a hepatospecific enzyme that is principally found in the cytoplasm in rats following the administration of CCl<sub>4</sub> is attributed to the increased release of the enzymes from damaged liver parenchymal cells. Similar results of ACP and ALP were found to increase in hepatotoxin animals.

In the present study concanavalin-A administered group showed significantly increased levels of AST, ALT, ALP (**Fig. 9**), direct and indirect bilirubin (**Fig. 10**). Amino transferase is an important class of enzymes linking carbohydrate and amino acid metabolism there by clearly establishing the relationship between the intermediates of the citric acid and amino acids. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver diseases. In case of liver damage with hepatocellular lesions and parenchymal cell necrosis, these enzymes are released from the damaged tissue into the blood stream. The decrease in alanine amino transferase activity is usually accompanied by lowering in the activity of aspartate amino transferase, in the present study

the activities of aspartate and amino transferase enzymes were maintained at near normal level in the oral administrated group of plant extract as compared with the hepatotoxin group. Alkaline phosphatase activity on endothelial cell surface is responsible for the conversion of adenosine nucleotide to adenosine, a potent vasodilator and anti inflammatory mediator that results from injury. So, following, accumulation of IL-6 can leads to the production of adenosine to alkaline phosphatase, this may be the reason for the increment in ALP in hepatotoxin group. Bilirubin is a major breakdown product that results from the

destruction of RBC which is removed from the blood by the liver through conjugation and secreted into bile usually becomes elevated as a result of decreased uptake by the liver, decreased conjugation, decrease secretion from the liver or blockage of the bile ducts that is caused in liver damage. The oral administration of plant extract and (positive drug) silymarin 100 mg/kg were significantly reduced the level of marker enzymes. The hepatoprotective activity of different doses was also observed and maximum activity of 80.90% was noticed with 300 mg/kg of the plant extract (**Table 6**).

**TABLE 6: EFFECT OF *SUAEDA MICROPHYLLA* ON HEPATOPROTECTION ON CONCAVALIN-A INDUCED HEPATOTOXICITY**

Treatments	Dose (mg/kg)	Hepatoprotection
Vehicle control	Nil	Nil
Con-A treated	Nil	Nil
Con-A + Silymarin	100	61.38
Con-A + Extract (75 mg/kg)	75	26.52
Con-A + Extract (150 mg/kg)	150	52.0
Con-A + Extract (300 mg/kg)	300	80.90

Histopathological scores (**Table 7**) showing the severity level of fatty changes and hydrophobic changes in concavalin - A treated rats but, mild level of fatty changes were observed with silymarin and extract (300 mg/kg) treated rats,

moreover focal necrosis were observed with severe level in concavalin - A treated rats but few level of necrosis were observed with extract and silymarin treated rats.

**TABLE 7: HISTOPATHOLOGICAL CHANGES IN THE LIVER OF THE RATS TREATED WITH SILYMARIN AND PLANT EXTRACT**

Treatments	Microscopic Observations					
	Degeneration in Hepatocytes (fatty and hydrophobic changes)	Deformation in Hepatocytes	Focal necrosis	Congestion in central vein	Congestion in sinusoids	Bleeding area in hepatic lobes
Vehicle Control	+	-	-	+	-	-
Con-A treated	++++	+++	+++	++	++	++
Con-A + Silymarin	+	⊥	⊥	+	⊥	⊥
Con-A + Extract 75 mg/kg	+++	++	++	++	++	+
Con-A + Extract 150 mg/kg	++	+	⊥	+	+	+
Con-A + Extract 300 mg/kg	+	⊥	⊥	++	+	⊥

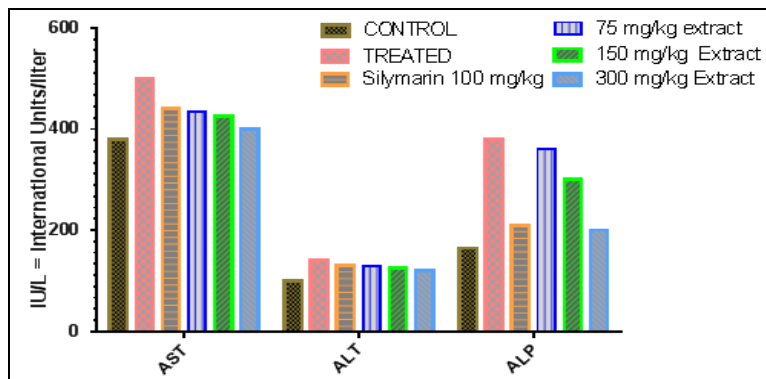
- Absent: ⊥ few: + mild: ++ moderate: +++ severe: ++++ extremely severe (the liver of 6 animals in every group were examined)

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoids space and proper central vein (**Fig. 11**). Whereas concavalin - A induced rats showed distorted sinusoids and hepatic necrosis (**Fig. 11b**). Rats pretreated with silymarin (100 mg/kg) and ethanolic extract (300 mg/kg) followed by concavalin - A hepatotoxicity showed a sign of protection as it was evident from the reduction of necrosis and normalization of

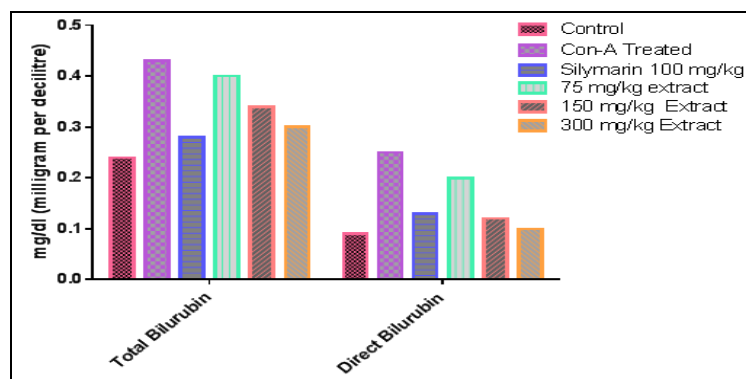
sinusoidal structure (**Fig. 11c- 11f**). Results of DPPH assay, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity and superoxide radical scavenging assay of plant extract and Vitamin C were represented in **Table 6**. It revealed that, hydroxyl radical scavenging ( $52.21 \pm 1.32 \mu\text{g/ml}$ ) and nitric oxide scavenging ( $09.14 \pm 0.94 \mu\text{g/ml}$ ) activity showed comparable activity with the standard Vitamin - C ( $44.24 \pm 1.50 \mu\text{g/ml}$  and  $4.98 \pm 1.28 \mu\text{g/ml}$ ).



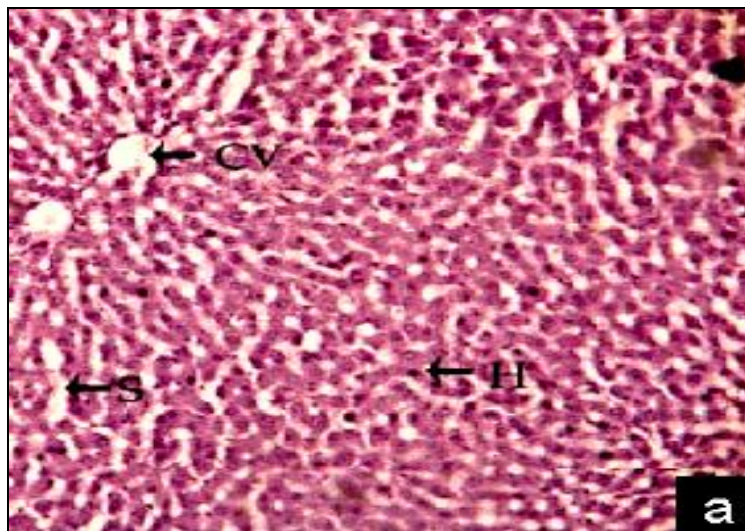
Previously, it was reported that, reactive oxygen species play a major role in Concanavalin - A induced hepatitis through secondary immune mediated liver damage and the hepatoprotective effect of *S. microphylla* leaf extract might be due to the presence of antioxidant properties.



**FIG. 9: EFFECT OF ETHANOLIC EXTRACT OF *SUAEDA MICROPHYLLA* AND SILYMARIN (POSITIVE CONTROL) ON SERUM LEVEL OF AST, ALT AND ALP (IU/L) DURING CONCAVALIN-A INDUCED HEPATOTOXIN RATS.** [Values are mean  $\pm$  S.D. (n=6). <sup>a</sup>Group II (concanavalin-A treated rats) compared with group I (control rats). <sup>b</sup>Group III, IV, V and VI (silymarin and plant extract treated rats) compared with Group II (concanavalin-A treated rats), <sup>c</sup> $P < 0.05$ ].



**FIG. 10: EFFECT OF ETHANOLIC EXTRACT OF *SUAEDA MICROPHYLLA* AND SILYMARIN (POSITIVE CONTROL) ON SERUM LEVEL OF TOTAL AND DIRECT BILURUBIN (mg/dl) DURING CONCAVALIN-A INDUCED HEPATOTOXIN RATS** [Values are mean  $\pm$  S.D. (n=6). <sup>a</sup>Group II (concanavalin-A treated rats) compared with group I (control rats). <sup>b</sup>Group III, IV, V and VI (silymarin and plant extract treated rats) compared with Group II (concanavalin-A treated rats), <sup>c</sup> $P < 0.05$ ].



**FIG. 11 (a): LIVER SECTION OF CONTROL RAT SHOWING NORMAL CELLULAR ARCHITECTURE WITH DISTINCT HEPATIC CELLS, SINUSOIDAL SPACES AND PORTAL VEIN**

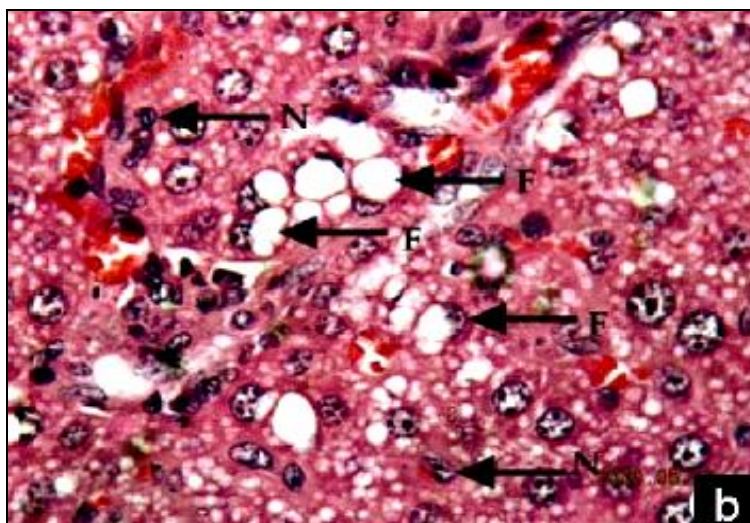


FIG. 11 (b): LIVER SECTION OF CONCANAVALIN-A RAT SHOWING DISARRANGEMENT AND REARRANGEMENT OF VACUOLED HEPATIC CELLS AND HEPATIC NECROSIS

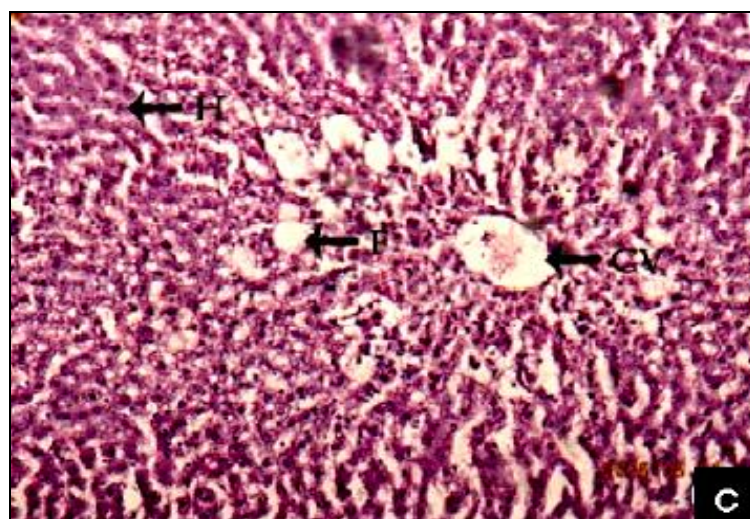


FIG. 11 (c): LIVER SECTION OF SILYMARIN TREATED RAT SHOWING LESS FATTY CHANGE FORMATION AND REDUCTION IN HEPATIC NECROSIS

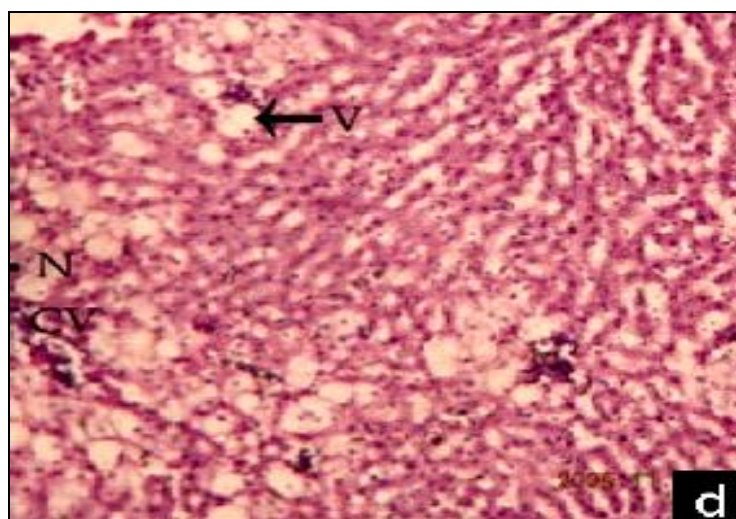


FIG. 11 (d): LIVER SECTION OF THE RAT TREATED WITH PLANT EXTRACT 75 mg/kg AND CONCANAVALIN-A, 100 ×, HAEMATOXILIN-EOSIN STAIN. LIVER SECTION OF THE RAT SHOWS FATTY CHANGE FORMATION AND HEPATIC NECROSIS

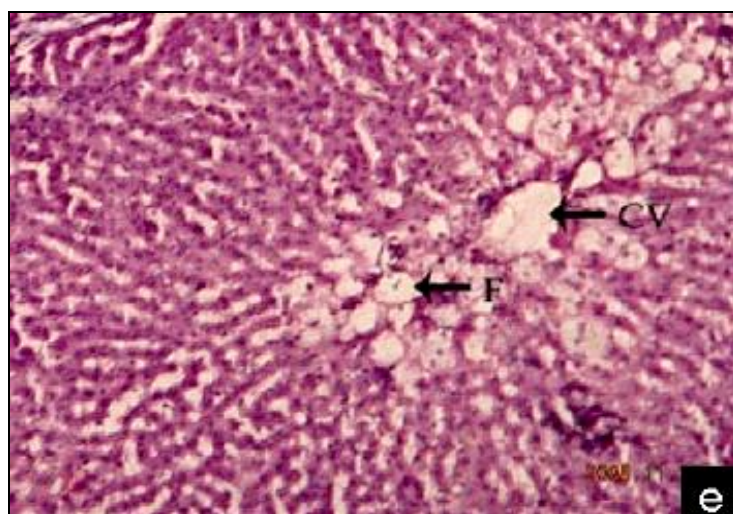


FIG. 11 (e): LIVER SECTION OF THE RAT TREATED WITH PLANT EXTRACT 150 mg/kg AND CONCANAVALIN-A, 100 $\times$ , HAEMATOXILIN AND EOSIN STAIN. LIVER SECTION OF THE RAT SHOWING REDUCTION IN FATTY CHANGE FORMATION AND HEPATIC NECROSIS

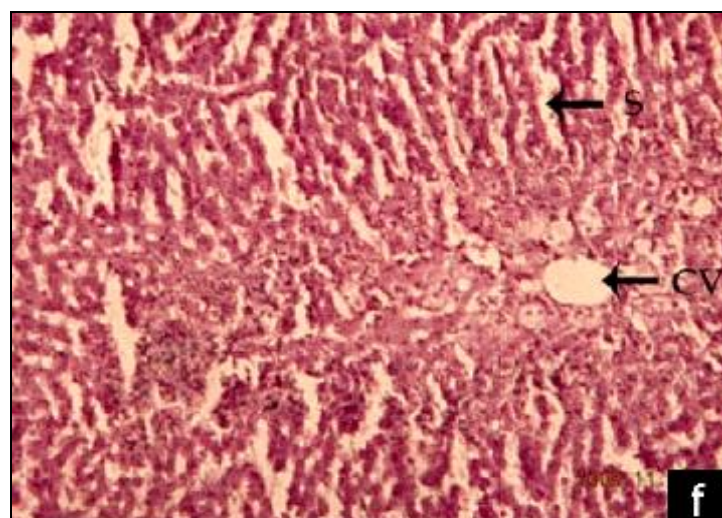


FIG. 11(f): LIVER SECTION OF THE RAT TREATED WITH PLANT EXTRACT 300 mg/kg AND CONCANAVALIN-A, 100 $\times$ , HAEMATOXILIN AND EOSIN STAIN. LIVER SECTION OF THE RAT SHOWING NORMAL ARCHITECTURE WITH LESS FATTY CHANGE FORMATION AND REDUCTION IN HEPATIC NECROSIS

H & E 100  $\times$  [CV: Central Vein; S: Sinusoidal spaces; H: Hepatocytes; F: Fatty changes; N: Hepatic necrosis]

TABLE 8: IC<sub>50</sub> VALUES OF *S. MICROPHYLLA* LEAF EXTRACT AND VITAMIN C WITH VARIOUS ANTIOXIDANT ACTIVITIES

Parameters	<i>S. microphylla</i> IC <sub>50</sub> ( $\mu$ g/ml)	Vitamin C IC <sub>50</sub> ( $\mu$ g/ml)
DPPH radical scavenging	91.70 $\pm$ 1.09	2.87 $\pm$ 1.26
Hydroxyl radical scavenging	52.21 $\pm$ 1.32	44.24 $\pm$ 1.50
Nitric oxide radical scavenging	09.14 $\pm$ 0.94	4.98 $\pm$ 1.28
Super oxide radical scavenging	145.25 $\pm$ 0.93	24.31 $\pm$ 0.71

#### Research Assay: 4

##### Hepatoprotective and antioxidant activity of methanolic extracts of *Sesuvium portulacastrum* (L.) against CCl<sub>4</sub> –induced liver injury in rats:

The methanol extract of whole plant of *Sesuvium portulacastrum* subjected for phytochemical study showed the presence of alkaloids, flavonoids,

Saponins, phenols, tannins carbohydrates, protein, amino acids, steroids, sterols, glycosides and terpenoids. The effect of methanol extract of *Sesuvium portulacastrum* on body weight of the normal, CCl<sub>4</sub> intoxicated and drug treated rats are shown in Table 9. Table 10 shows the effect of methanol extract of *Sesuvium portulacastrum* on

serum total protein, albumin, globulin, A/G ratio, serum transaminases, and alkaline phosphatase in CCl<sub>4</sub> intoxicated rats. There was a significant (p<0.01) increase in serum GOT, GPT and ALP levels in CCl<sub>4</sub> intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels were significantly (p<0.01) decreased to 6.93g/dl and

4.11g/dl in CCl<sub>4</sub> intoxicated rats from the levels of 8.58g/dl and 4.93g/dl respectively in normal group. Methanol extract of *S. portulacastrum* at the dose of 300mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

**TABLE 9: EFFECT OF *SESUVIUM PORTULACASTRUM* EXTRACT ON THE BODY WEIGHT OF IN THE NORMAL, LIVER DAMAGED AND DRUG TREATED RATS**

Treatment	Dose	Initial body weight(g)	Final body weight(g)	Mean weight gain (G ↑) /loss(L ↓) (g)	% Difference
Group I	0.9% Saline	156.84±4.39	188.36±3.93	29.52 ↑	18.58
Group II	0.9% Saline	190.55±3.86	171.65±4.30	21.90 ↓	11.31
Group III	150(mg/Kg)	170.46±4.33	163.11±3.54**	8.35 ↑	4.81
Group IV	300(mg/Kg)	196.51±6.28	191.61±4.25ns a	5.90 ↑	2.90
Group V	100(mg/Kg)	185.25±4.34	192.54±4.88nsa	8.29 ↓	4.40

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*P <0.05,\*\*P <0.01 as compared with Normal Control to liver damaged control NS: not significant

**TABLE 10: EFFECT OF *SESUVIUM PORTULACASTRUM* EXTRACTS ON THE SERUM PROTEIN, ALBUMIN, GLOBULIN CONCENTRATION AND SERUM GOT, GPT AND ALP ENZYME ACTIVITY IN THE NORMAL, LIVER DAMAGED AND DRUG TREATED RATS**

Groups	Parameters							
	Dose	T.Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group I	0.9% Saline	8.58±1.65	4.93±0.17	3.65±0.12	1.3:1	12.63±0.58	19.46±0.27	181.63±4.23
Group II	0.9% Saline	6.93±0.93*	4.11±0.34	2.82±0.11*	1.4:1	43.92±0.63**	53.63±0.38**	248.74±6.85**
Group III	150(mg/Kg)	7.21±0.78ns	4.08±0.16	3.13±0.62	1.3:1	40.66±0.38**	42.14±0.13*	211.86±5.69*
Group IV	300(mg/Kg)	8.66±0.36aa	4.32±0.83	4.34±0.13	1.0:1	18.14±0.21*aa	26.81±0.85ns	192.84±4.15
Group V	100(mg/Kg)	8.36±0.38aa	4.71±0.11	3.65±0.18	1.3:1	13.99±0.18aa	18.26±0.22aa	173.16±2.65aa

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*P <0.05,\*\*P <0.01 as compared with Normal Control to liver damaged control: a P<0.05; aa P<0.01 aaaP<0.001 as compared with liver damaged control to drug treated animal NS: not significant.

The effect of methanol extract of *Sesuvium portulacastrum* on total, conjugated and unconjugated bilirubin is shown in **Table 11**. A significant elevation of total, conjugated, unconjugated bilirubin and  $\gamma$ -glutamyltransferase in the serum of CCl<sub>4</sub> intoxicated group (Group II) when compared to normal control (Group I). The methanol extract of *Sesuvium portulacastrum* at the dose of 150mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and  $\gamma$ -glutamyltransferase were found to be greater in standard Silymarin (Group V) followed by Group II and Group III (**Table 11**).

The effects of methanol extract of *Sesuvium portulacastrum* on lipid peroxidation (LPO), Glutathione peroxidase (GPX), glutathione

reductase (GRD), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activity is shown in **Table 12**. Lipid peroxidation level was significantly (p<0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p<0.01) decreased in CCl<sub>4</sub> intoxicated rats when compared with those of the animals in normal control group. Rats treated with methanol extract of *Sesuvium portulacastrum* at the doses of 150mg/kg significantly decreased the elevated lipid peroxidation levels and restored, the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and reduced glutathione levels towards the normal levels in a dose dependent manner. The results are well comparable with Silymarin (standard drug) treated group.

**TABLE 11: EFFECT OF *SESUVIUM PORTULACAISTRUM* EXTRACTS ON THE SERUM TOTAL, CONJUGATED, UNCONJUGATED BILURUBIN AND GGTP LEVELS IN THE NORMAL CONTROL, LIVER INJURED AND DRUG TREATED RATS**

Groups	Parameters				
	Dose	Total Bilurubin (Mg/dl)	Conjugated (Mg/dl)	Unconjugated (Mg/dl)	GGTP (U/L)
Group I	0.9% Saline	0.84±0.12	0.28±0.17	0.56±0.14	6.23±0.94
Group II	0.9% Saline	4.38±1.13**	3.65±0.94**	0.73±0.12*	21.84±1.08**
Group III	150(mg/Kg)	2.56±0.84*	1.73±0.39*	0.83±0.15*	16.55±0.85ns
Group IV	300(mg/Kg)	2.03±0.75nsa	1.29±0.21*	0.74±0.11ns	11.84±0.21a
Group V	100(mg/Kg)	1.84±0.22aa	1.03±0.17aa	0.81±0.21	7.33±0.21aa

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*P <0.05; \*\*P <0.01as compared with Normal Control to liver damaged control: a P<0.05; aa P<0.01 as compared with liver damaged control to drug treated animal NS: not significant

**TABLE 12: EFFECT OF *SESUVIUM PORTULACAISTRUM* ON SERUM LPO, GPX, GRD, SOD, CAT AND GSH ACTIVITY IN THE NORMAL CONTROL, LIVER INJURED AND DRUG TREATED RATS**

Groups	Parameters						
	Dose	LPO (n mole of MDA/mg protein)	GPX (u/mg Protein)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
Group I	0.9% Saline	2.635±0.014	4.934±0.081	0.409±0.073	0.324±0.024	3.911±0.014	31.94±0.24
Group II	0.9% Saline	5.081±0.051**	1.253±0.024**	0.196±0.055**	0.126±0.018**	1.094±0.012**	9.63±0.18**
Group III	150(mg/Kg)	4.113±0.049*	1.941±0.073*	0.312±0.038*	0.248±0.021*	1.984±0.034*	15.84±0.26*
Group IV	300(mg/Kg)	2.84±0.024ns	3.08±0.018a	0.394±0.014nsaa	0.294±0.017nsaa	2.534±0.017	26.33±0.75*aa
Group V	100(mg/Kg)	2.926±0.016aa	4.683±0.075aa	0.398±0.014aa	0.343±0.079aa	3.584±0.013aa	27.14±0.68

Values are mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. \*P <0.05; \*\*P <0.01 as compared with Normal Control to liver damaged control: a P<0.05; aa P<0.01 as compared with liver damaged control to drug treated animal NS: not significant.

The present study provides scientific evidence on the correlative effect of antioxidant and hepatoprotective activities. Elevation of serum markers are a known effect of CCl<sub>4</sub> toxicity and used as biochemical parameters of liver damage. The toxicity produced by CCl<sub>4</sub> is mediated through free radical mechanism. CCl<sub>4</sub> is metabolized by cytochrome P450 enzyme and its metabolic products, trichloromethyl free radicals that are highly reactive and induced lipid peroxidation of macro molecules leading to tissue injury. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals.

The extent of hepatic damage was assessed by histological evaluation and level of various biochemical parameters. From the results of the present investigation it was evident that the methanol extracts of *Sesuvium portulacaistrum* were able to reduce the hepatotoxin intoxication induced elevated biochemical parameters. Assessment of liver toxicity was done by measuring the marker enzymes such as SGOT, SGPT and ALP which are originally present in high concentration in the cytoplasm. When there is hepatic injury these enzymes leak into blood

stream inconformity with extent of hepatotoxicity treatment with methanol extracts of *Sesuvium portulacaistrum* restored the elevated levels of serum marker enzymes. The normalization of serum markers by *Sesuvium portulacaistrum* whole plant suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl<sub>4</sub> induced leakages of marker enzymes into the circulation.

Protein metabolism is a major function of liver and a healthy functioning liver is required for the synthesis of the serum protein. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. The reduction in the serum albumin and globulin levels in CCl<sub>4</sub> intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthesis function of the liver. Treatment with methanol extract of *Sesuvium portulacaistrum* whole plant ameliorated the imbalance. Serum bilirubin in is one of the most sensitive tests employed in the diagnosis of hepatic disease. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract, there is a mass inhibition of the conjugation

reaction and release of unconjugated bilirubin from damaged and dead hepatocytes. Administration of *Sesuvium portulacastrum* decreases the level of bilirubin and increased the level of protein suggesting that it offered protection.  $\gamma$ -glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. It is most useful in the diagnosis of liver diseases. The acute damage caused by  $\text{CCl}_4$  increased the  $\gamma$ -glutamyltransferase level but the same attains the normal after *Sesuvium portulacastrum* treatment due to its antioxidant activity.

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated to the destructive process of liver injury due to acetaminophen administration. In the present study the elevations in the levels of end products of lipid peroxidation in the liver of the rat treated with  $\text{CCl}_4$  was observed. The increase in Malondialdehyde (MDA) levels in liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *Sesuvium portulacastrum* whole plant significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection by methanol extract of *Sesuvium portulacastrum* whole plant due to its antioxidant effects.

Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon,

liver, lymphocytes and brain. The elevation of MDA level, which is one of the end products of lipid peroxidation in the liver tissue, and the reduction in hepatic GSH levels are important indicators in  $\text{CCl}_4$  intoxicated rats. In this study, it was ascertained that MDA levels have been suppressed compared to  $\text{CCl}_4$  intoxicated group and  $\text{CCl}_4$  induced depletion of GSH was prevented.

Superoxide dismutase (SOD), a metalloprotein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the methanol extract of *Sesuvium portulacastrum* whole plant significantly increased the SOD activity in  $\text{CCl}_4$  intoxicated rats thereby diminished  $\text{CCl}_4$  induced oxidative damage.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found to the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Therefore the reduction in the activity of these enzymes may result in the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of methanol extract of *Sesuvium portulacastrum* increased the activities of CAT in  $\text{CCl}_4$  induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from  $\text{CCl}_4$  intoxication. Glutathione peroxidase (GPX) is a seleno enzyme, it protect the cells from damage due to free radicals like hydrogen and lipid peroxide. It catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulphide and reduction.

**CONCLUSION:** DNA barcoding is an essential tool to identify correct taxonomic sequencing either in plants and animals. DNA barcoding studies on some of the fragile Mangrove Plants are initiated to document the identity at nucleotide level. It is easy to identify the most variable

regions in certain taxa at species level; however, it is difficult, and even unlikely, to identify such regions in all taxa. Currently, there are two applications for DNA Barcoding. One application is for flora, and other application is for specific taxa. The application is exemplified by barcoding tree in large ecological plots, and the similar example was given in this study.

Our test example differs from the barcoding of local flora in that more species are from same genera, which may show reduced discriminating power. According to current findings compared to chloroplast genes like *rbcL*, *matK* and *trnH-psbA*, and the nuclear internal transcribed spacer (ITS), the *ycf1a* and *ycf1b* regions are perhaps the most variable regions in most taxa. A barcode should be chosen because it shows the highest species resolution in most cases rather than in specific cases. The *ycf1* genes meet this criterion and can serve as a barcode of land plants.

In conclusion, this study provides preliminary assessment data that will be useful for wider application of DNA Barcoding in ecological studies of Mangrove Plants. With the current development of primers the *rbcL* is very useful for the barcoding of plant species. However further protocol development to enhance clean DNA extraction, PCR amplification strategies, including the development of new primers and local authenticated data bases would play important roles in efficient utilization of plant barcoding.

Biological assessment data accuracy which will be useful for proper utility of its compounds in the food and pharmacopeia industries. Phytochemical Qualitative analysis of *S. prostrata*, *S. microphylla* and *S. portulacastrum* leaves showed that the presence of biologically important phyto-constituents viz., Flavanoids, Terpenoids, Tannins, Phenols and Cardiac glycosides. This research work has revealed the potential of three mangrove species could be useful in the area of life sciences. Further research work is need to determine the biological active molecules from *S. prostrata*, *S. microphylla* and *S. portulacastrum*, this would be the basic platform to be executed in various applications of life sciences. It is concluded that, administration of concavalin-A resulted in a

significant increase in the AST, ALP, ALT and bilirubin level and these changes in the marker levels reflected in hepatic structural integrity. Retreatment with alcoholic extract of *Suaeda microphylla* attenuated the elevated level of serum markers. Normalization of serum markers by ethanolic extracts suggested that, hepatocytes are conditioned to protect the membrane integrity against concavalin-A induced leakage of marker enzymes into the circulation. Elevated level of bilirubin indicates the hepatotoxicity. The normalization of the blood bilirubin extract in pretreated rats further indicated the protective nature of the extract on hepatic cells.

Histopathological examination of liver sections revealed that, the normal liver architecture was distributed by hepatotoxin intoxication. Sections obtained from the plant extract and silymarin groups showed normal cell architecture, although less visible changes were observed which further corroborate the hepatoprotective activity. The hepatoprotective activity of *Suaeda microphylla* may be due to the presence of triterpene phytochemical constituents. Hence, triterpenes are proved to have hepatoprotective activity. Further studies are required to identify the active principles present and to establish the mechanism of action. The results of this study demonstrate that *S. portulacastrum* has potent hepatoprotective action upon carbon tetrachloride induced hepatic damage in rats.

Our result showed that the hepatoprotective effect of *Sesuvium portulacastrum* whole plant may be due to its antioxidant and free radical scavenging properties. Hepatoprotective activity of *Sesuvium portulacastrum* whole plant may be due to the presence of tannins, terpenoids, flavonoids, alkaloids, saponins and phenols. *Sesuvium portulacastrum* whole plant methanolic extract has contributed to the reduction of oxidative stress and showed hepatoprotective activity in experimental rats.

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