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Isolation of Phytoconstituents From *Streblus Asper* Flor and Its Docking Study for Few Pharamacological Actions

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ABSTRACT.

Streblus asper (family-Moraceae) is a little tree which is local to tropical countries, for instance, India, Srilanka. Philippines and Thailand. In India it is appropriated in the Himalayas from Himachal Pradesh to West Bengal and in inclines and fields of Assam and Tripura. It is similarly found in the drier bits of India. Various bits of this plant are used in Ayurveda and other society prescription for the therapy of different infections, for instance, Filariasis, Disease, Tooth hurt, Diarrhea, Dysentery, and Cancer. Root is used as application to the unfortunate ulcers and sinuses, and as counteractant to snakebite, in epilepsy and weight. Stem is used in toothache, Stem bark is yielded fever, detachment of the guts, the runs, stomach pulsate, urinary complaints, loads, edema and wounds.This study considered about the innate science, science, traditional uses, pharmacological development and various works that are passed on reliant upon this drug and major mark of these review is to aggregate invigorated information on the prescription *Streblus asper* lour for its more forward-thinking improvement of substance constituents by using various solvents like ethanol and acetone for extraction of constituents and examination by using distinctive made procedures like lc-ms, proton and carbon nmr, IR spectroscopy and docking studies was performed for already developed to determine antioxidant and cancer activity.

Keywords: - *Streblus asper*, spectroscopy, anticancer, antioxidant activity, docking studies. Corresponding author

1. INTRODUCTION

Streblus asper Lour which has a place with Moraceae family is a little tree which is local to tropical nations like Philippines, Malaysia, srilanka and India2. It is an unbending bush or twisted tree; branchlets tomentose or pubescent. Leaves are 2–4 inch, unbending, elliptic, rhomboid, applaud or obovate, unpredictably toothed; petiole 1/12 inch. Male heads globose, single or 2-Nate, some of the time male/female; peduncle short scabrid, blossoms minute[2]. Female blossoms longer peduncled. Natural product pisiform; perianth yellow and it is a plant called by various vernacular names like Rudi, Sheora, Koi, Siameseroughbush and Tooth brush tree etc[3] .

Fig 1:- *Streblus asper* plant

Medicinal plants have been widely used in traditional system to treat several diseased conditions in the world. Hence therefore it became necessary to carry out the estimation of safety and effectiveness of chemical constituents that have been extracted. In ancient days it was used as tooth brush due strengthing effect of gums and teeth and also the various parts of the plant like extract of roots reported against cardiac activity, extract of leaves reported against epilepsy, leprosy, extract of stem reported as antimicrobial ,antibacterial[4]. The various chemical constituents reported were glycosides like cardiac glycosides, steroidal glycosides, terpenoids etc. This review is concerned about the isolation and extraction of phytoconstituents, pharmacological activity of the selected plant *etc*. Hence pharmacogonsy of the plant on

different parts has been carried out. All the properties are filed and updated information as well gathered to complete research study on *S.asper* up to date [5, 6].
Therefore this research is concerned about compiling of work from the beginning till to date and provided a new way outlet to carry out further research on the above mentioned drug in the figure 2 and 3

Fig:-2 S.asper root

Fig:-3 S.asper stem

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational drug design[7].
Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation is used to predict the strength of association or binding affinity between two molecules using scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids carbohydrates, and lipids play central role in signal transduction. Therefore, docking is useful for predicting both the strength and type of signal produced. Docking is frequently used to predict the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational designof drugs[8].

2. EXPERIMENTAL SECTION.

CHEMICALS

Acetone, Hexane, Methanol, ethanol, dicholromrthane,ethylacetate, Benedict's reagent, Sodium Hydroxide, Copper Sulphate, Hydrochloric Acid, Dragondorff´s Reagent, Ferric chloride, Sodium Chloride, Lead Acetate, Chloroform, Concentrated Sulphuric Acid, Acetic Acid. Silica gel GF, etc all used chemicals were Laboratory graded.

COLLECTION OF PLANT MATERIALS

Stem and root of *Streblus asper* has to be authenticated by an expert botanist, a good specimen from the collected plant material, which is having good phytoconstituents and collected parts were washed with water, then dried, then washed with alcohol, and kept for drying by pressing method and prepared the herbarium. Plant materials was identified and authenticated by Dr. K. Madhava chetty (Plant taxonomist) Assistant professor, Department of Botony, Sir Venkateswara University, Tirupati, India The samples were dried after cleaning and powdered using grinder to reduce the size of the drug taken inoder to enhance the extraction process.

DRYING AND PULVERIZATION PROCESS.

The required plant material was collected and washed with tap water. Cleaned stem and root parts were allowed for air dry. The collected plant material was pulverized or granulized (It is a process or method followed in order to reduce the size of plant material into coarse powder to process for further analysis)[9]. The collected material was stored in air tight container inorder to avoid contamination or escape of phytoconstitunets from the material and it was processed for further pharmacognostic analysis like extraction, chromatography, followed by spectroscopy like Infrared spectroscopy, liquid chromatography merged with mass spectroscopy[10-13].

PREPARATION OF EXTRACTS

20 g (Each 10 g mixture of stem and root) was taken and performed analysis called maceration that is nothing but determining about the percentage solubility of phytoconstituents in the selected solvents like Acetone, Ethanol, Water were maceration process gave an information that mixture of plant material as maximum solubility in Acetone and ethanol[14] .Hence mixture of solvent is used for further extraction process. The obtained extract was further processed for phytochemical analysis.

PHYTOCHEMICAL ANALYSIS.

The determination of physicochemical parameter was important in determination of adulterants and improper handling of drugs. The extract was analyzed for physicochemical characteristics. The observed parameters were recorded as shown in table 1 Different ash value was also determined on the residue of *Streblus asper* and reported. Ash values were important quantitative standards and criterion to judge the identity and purity of crude drugs especially in the powder form. Moreover the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug[15].

Sl.no	TESTS	OBSERVATIONS
1.	Nature	Powder
2.	Colour	Yellowish brown
3.	Odour	Characteristic.
4.	Taste	Characterstics
5.	Foreign matters	Nil
6.	Fluorescence analysis	Fluorescence
7.	Total moisture content	8.9-9.5%
8.	Total ash values (% w/w)	2.95

TABLE: - 01 PHYTOCHEMICAL ANALYSIS.

4. RESULT AND DISCUSSION.

Phytochemical screening for aceto-methanol extract of *Streblus asper* showed the presence of Carbohydrat flavonoids, alkaloids, phenolic compounds, tannins, glycosides, reducing sugar Acidic compounds, resins and saponis while, it gave negative results for acidic compounds,and anthraquinones.

ISOLATION OF ACTIVE CONSTITUENTS FROM THE EXTRACT:

The drug was processed for soxhelate extraction and the isolation of phytoconstituents by using 1:1 ratio solvent of acetone and methanol were the temperature was maintained about 55-60° c for 24hours and the obtained solvent was concentrated to get an extract.the obtained extract was than processed for detection of new mobile phase for the isolation of phytoconstituents.

DETECTION OF MOBILE PHASE:

About 50 TLC plates were eluted using different solvent in which the TLC plates showing number of bands (chemical compounds) for each fraction, that can be further isolated and purified using Column Chromatography (CC). Out of 50 TLC plates using different solvents (single solvent and in combination), 2 best TLC plates with good separation were selected for carrying out the Column Chromatography. The selected mobile phase with good separation compare to other mobile phase are found to be TLC plate No. 30 Chloroform; petroleum ether : benzene (4:3:3) and for TLC plate No. 32 as shown the maximum number spots that has been isolated. Hence, this solvent is used for further isolation of components with the help of column chromatography.

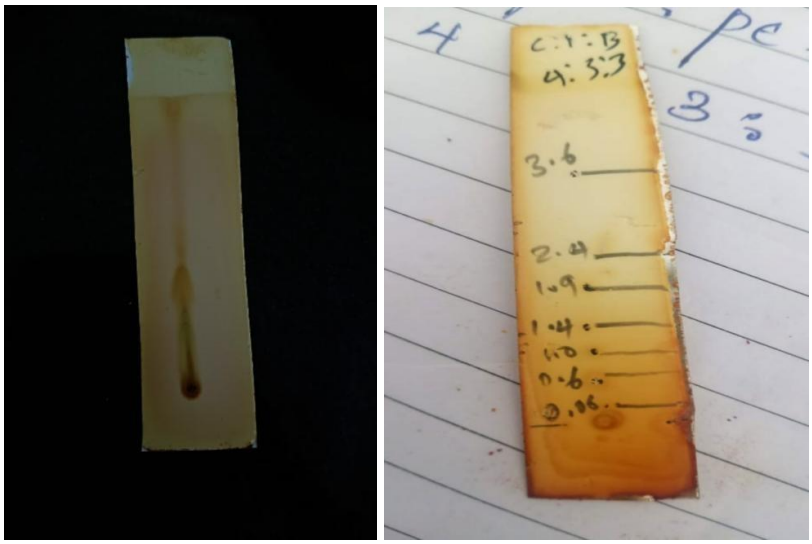


Fig 4:- TLC Plate No 30 and 32 with Good Separation.

Column chromatography:

The same solvent system which found by TLC chromatography, chloroform: petroleum ether benzene (4:3:3) (Figure 17) were used for column chromatography. After running the column the fractions were collected and checked for its *Rf* value by TLC. Fractions CCF01-12 has not shown any spot isolation or *Rf* value and CCF13- 17 were shown *Rf* value (0.055 g) which are not identical to the TLC plate Rf value even fractions CCF19-24 (0.144 g) as not shown any *Rf* value. Fractions CCF21-27 (0.467 g) were showed spots which were similar to the Rf values that obtained by TLC plate this shows that identical fractions has been eluted from the column chromatography.

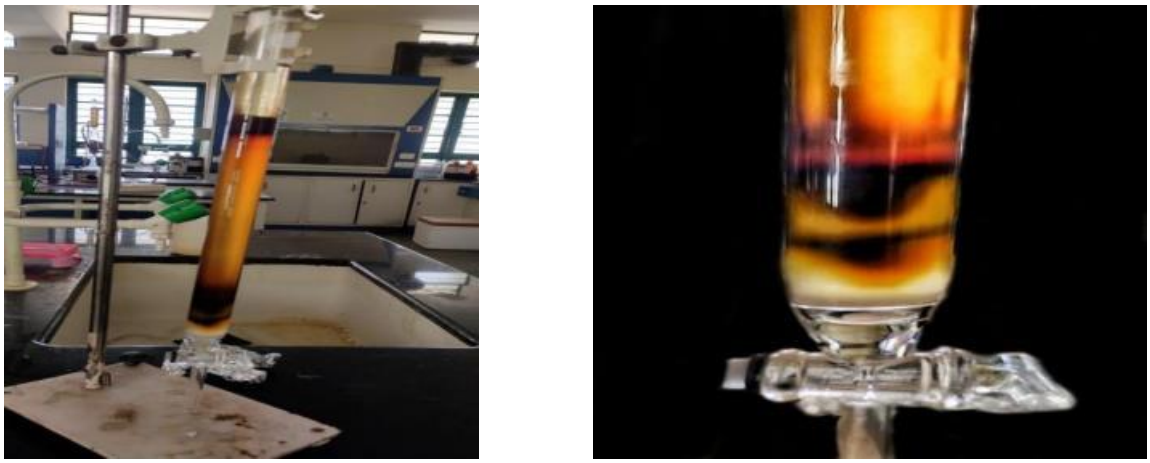


Fig 5:- Fractions of column chromatography

IR SPECTROSCOPY

IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances.

IR spectra of all fractions were checked, and the result shows that many fractions are having similar IR peaks and FTIR a rapid and nondestructive method that can detect a range of functional groups that are present in fractions that are isolated FTIR provides information on the basis of chemical composition and the physical state of liquid. The IR spectroscopy of fraction is mentioned in the below figure 6 and 7.

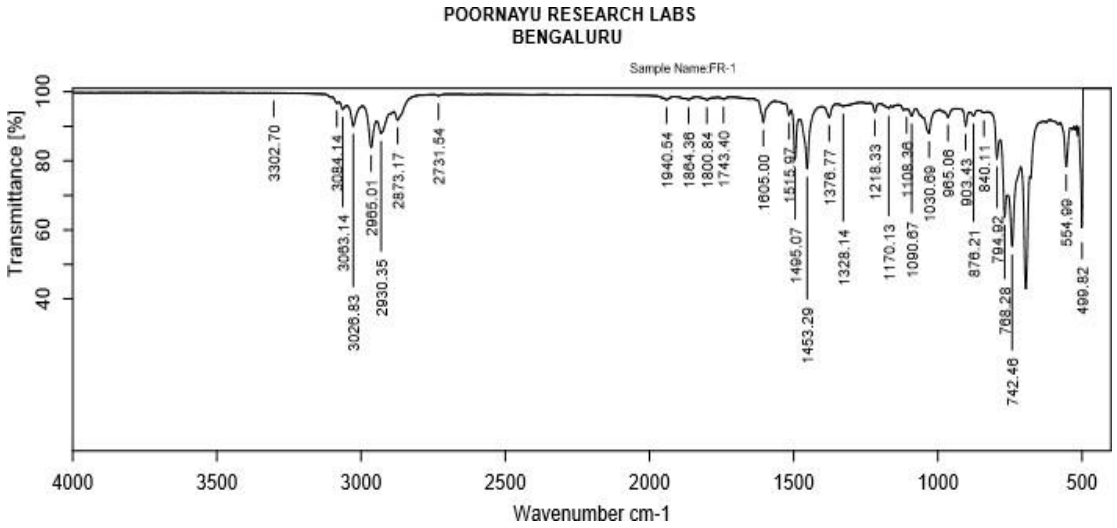


Fig 6:- IR spectra of fraction CCF22.

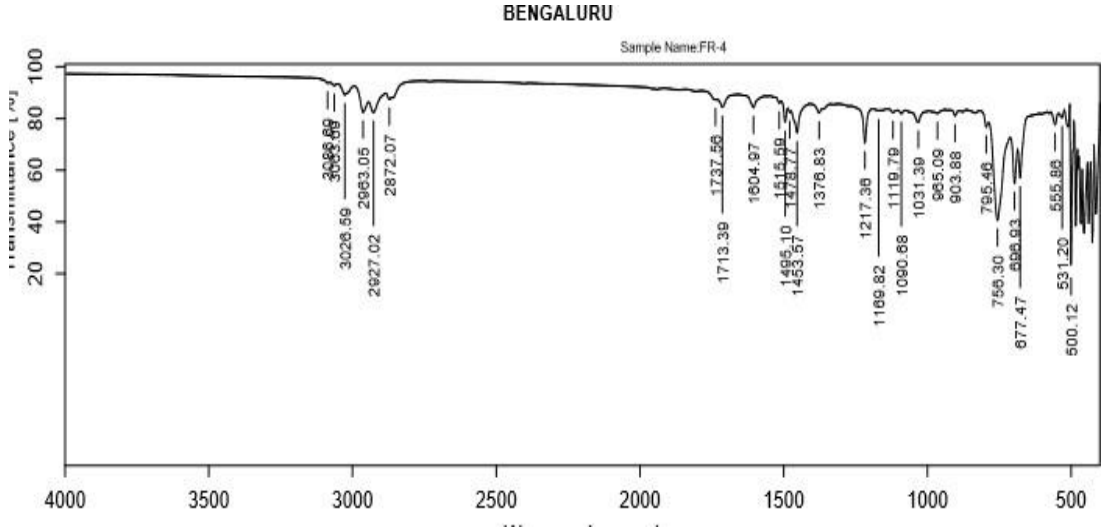


Fig 7:- IR spectra of fraction CCF22.

PROTON AND CARBON NMR SPECTROSCOPY.

The obtained fractions were further processed for NMR spectroscopy to study the proton and carbon nmr in order to study about the fraction and its arrangements which are shown in the respective figure 8 and 9.

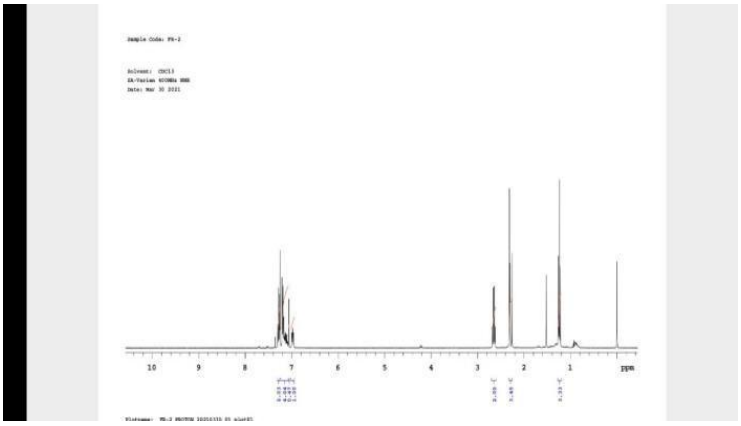


Fig 8:- Proton spectroscopy of CCF22

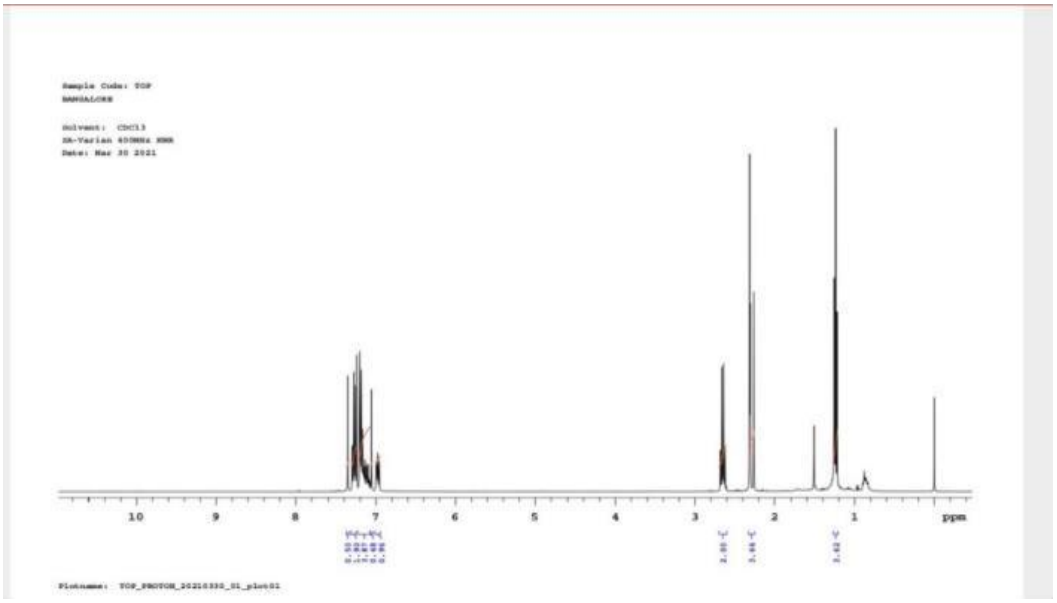


Fig 9:- Proton spectroscopy of CCF24

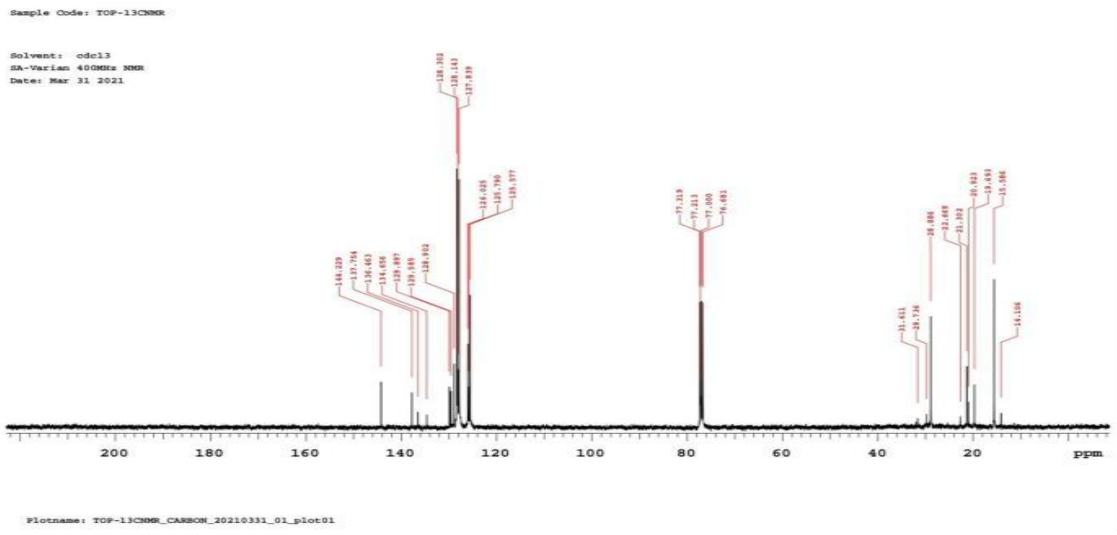


Fig 10:- Carbon spectroscopy of CCF24

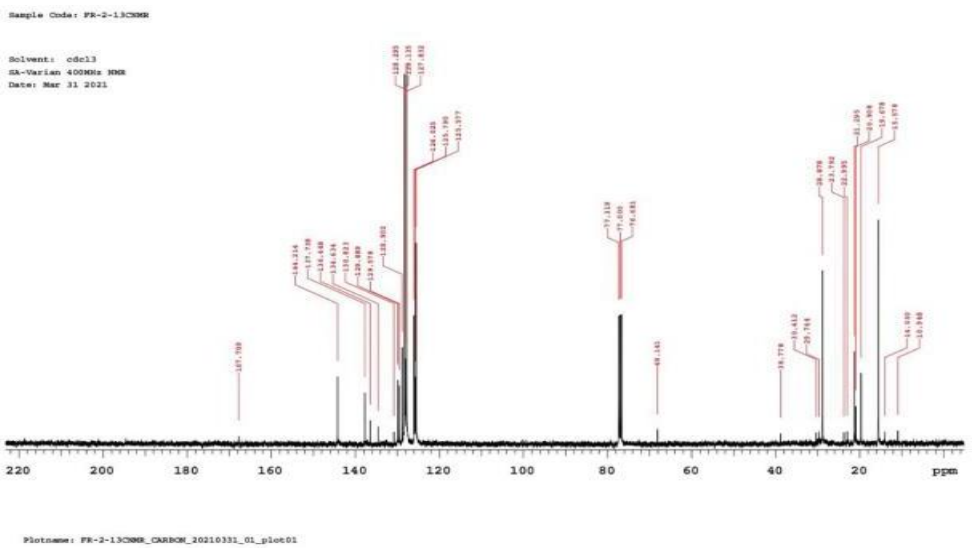
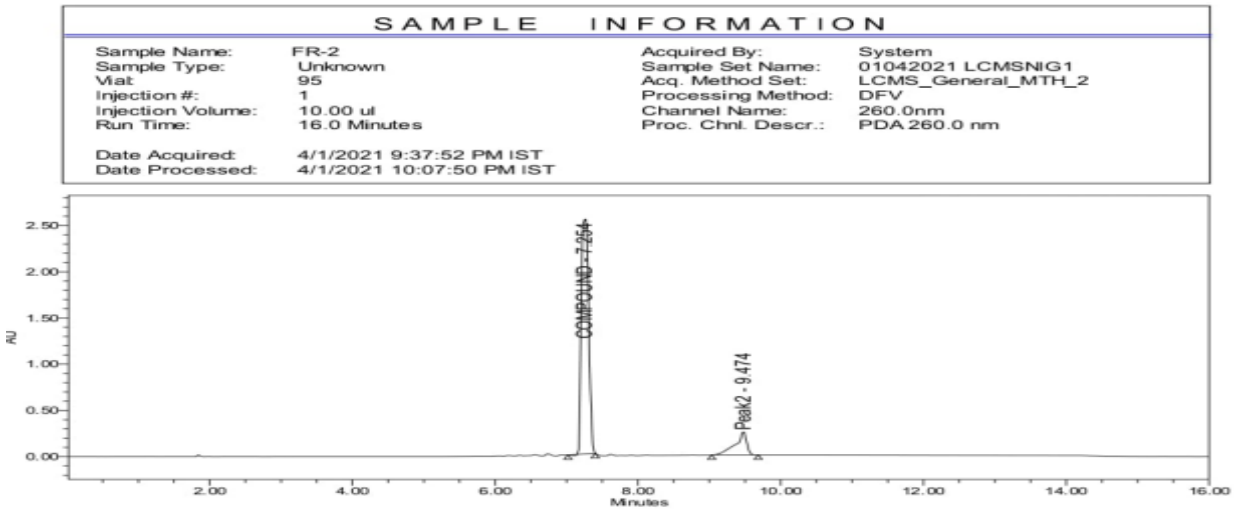


Fig 11:- Carbon spectroscopy of CCF22

LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY.

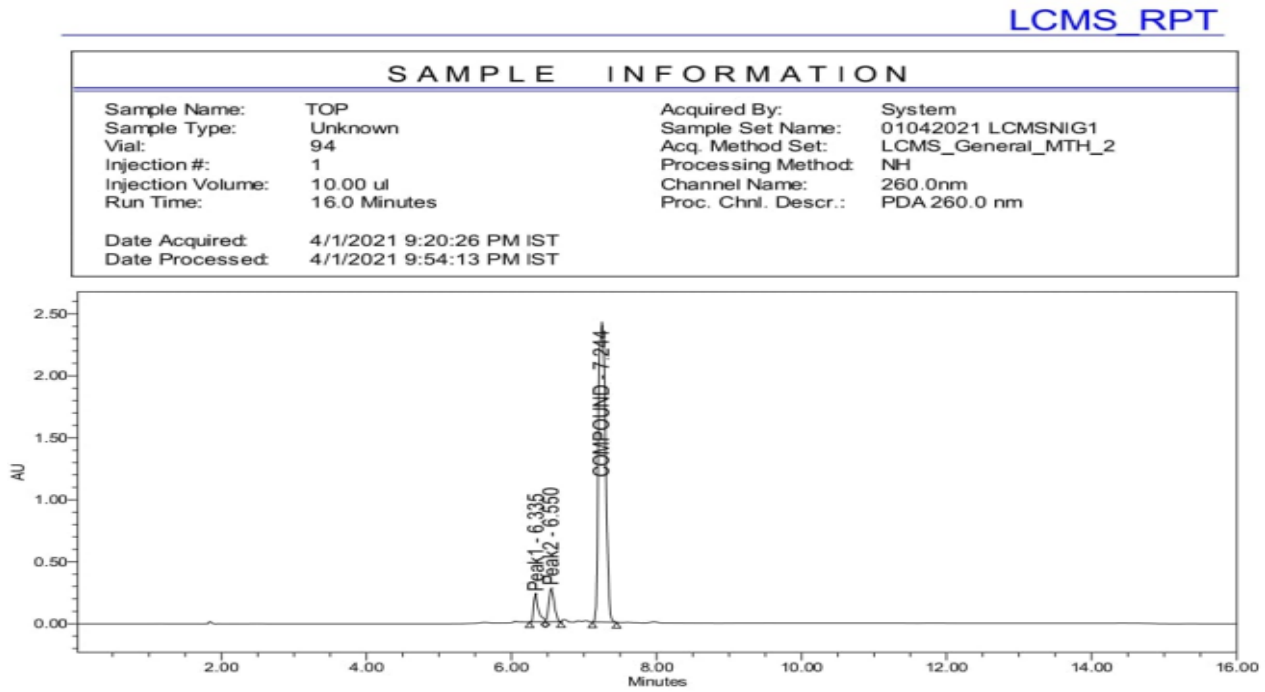
Technique that combines about resolving power of liquid chromatography with the detection specificity of mass spectroscopy where liquid chromatography separates the sample components and then introduces them to the mass spectrometer than MS creates and detects charged ions hence the fractions 22 and 24 was further processed for LC-MS analysis and obtained spectra's are attached below.



Peak Results

	Peak Name	RT	Area	% Area	RT Ratio
1	COMPOUND	7.254	18571806	87.17	
2	Peak2	9.474	2734338	12.83	1.306

FIG 12:- LCMS OF CCF 22.



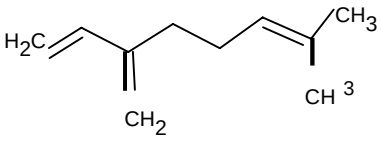
Peak Results

	Peak Name	RT	Area	% Area	RT Ratio
1	Peak1	6.335	1008389	5.31	0.874
2	Peak2	6.550	1402734	7.38	0.904
3	COMPOUND	7.244	16588070	87.31	1.000

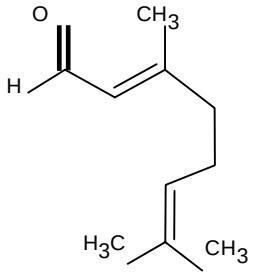
FIG 13:- LCMS OF CCF 24

The obtained fractions are further processed for analysis that is elucidation of structures with the help of obtained information and can be processed for determination of pharmacological activity.

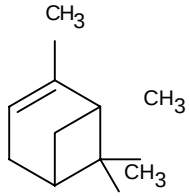
Based on obtained information the spectral analysis gave information that the selected solvent system can also able to isolate chemical constituents that is volatile oils from the plant crude. The obtained spectra are predicted to be some of the volatile constituents like CITRAL, PINENE, TERPINENE, P-CYMENE, SABINENE, CAMPHENE, and BETA-OCIMENE where this constituents are already proven for pharmacological activity of their own



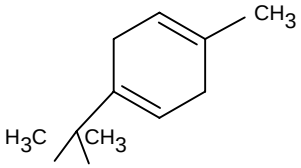
Structure 1:- Myrcene



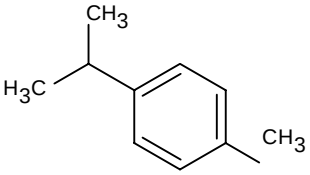
structure 2:- Citral



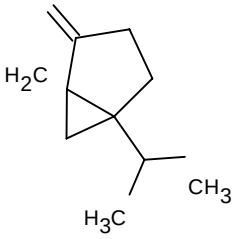
Structure 3:-Pinene



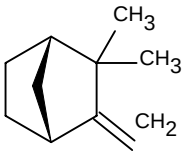
Structure 4:- TERPINENE



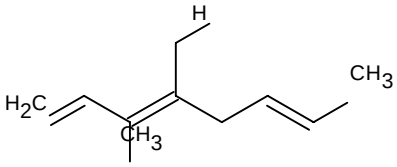
Structure 5:- p-cymene



Structure 6:- sabinene



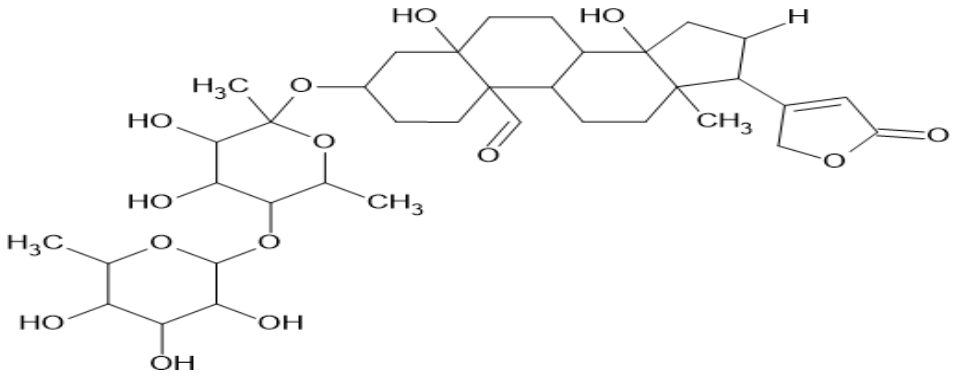
Structure 7:- Camphene



Structure 8:- Beta-Ocimene

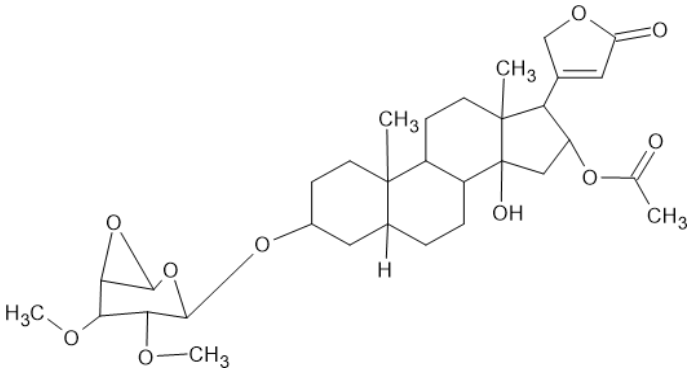
DOCKING STUDIES

The other part of the research was to determine the pharmacological activity of the compound or structure tht are already isolated .in this case I sincerely thank to Dan miao for the work he has done and for providing route for other research to carry out work from his work already isolated structures were taken and performed docking studies to determine the pharmacological activity[16-17].



STRUCTURE 9:- Strophanthidin-3-0-α-l-rhamnopyranosyl-1-4-6-deoxy

-β-acetylkamaloside



STRUCTURE 10:- 5Bh-16β-acetylkamalosid

In the selected structure the active site amino acids were selected for the binding energy determination.

Active site amino acids

Ala17, Thr18, Ala21, Arg22, Gly23, Val25, Glu26, Ala30, Glu33, Asn42, Tyr44, Gln50, Val51, Met54, Gly55, Ala57, Arg58, Leu62, Leu65, Asp74, Ala76, Leu78, His83, Asp84, Arg87, Glu88, Gly89, Asp92, Val96, Arg99, Asp108

The above mentioned sites are the active aminoacids of the taken two strutcures and was processed for docking by the cyclene depedent kinase inhibitor (CDK2A) which is present in all part were antioxidant preferative is present ,which act through PIK3 signaling pathway and no of hydrogen present in each structure along with binding acivity is described below.

ANTI CANCER ACTIVITY.

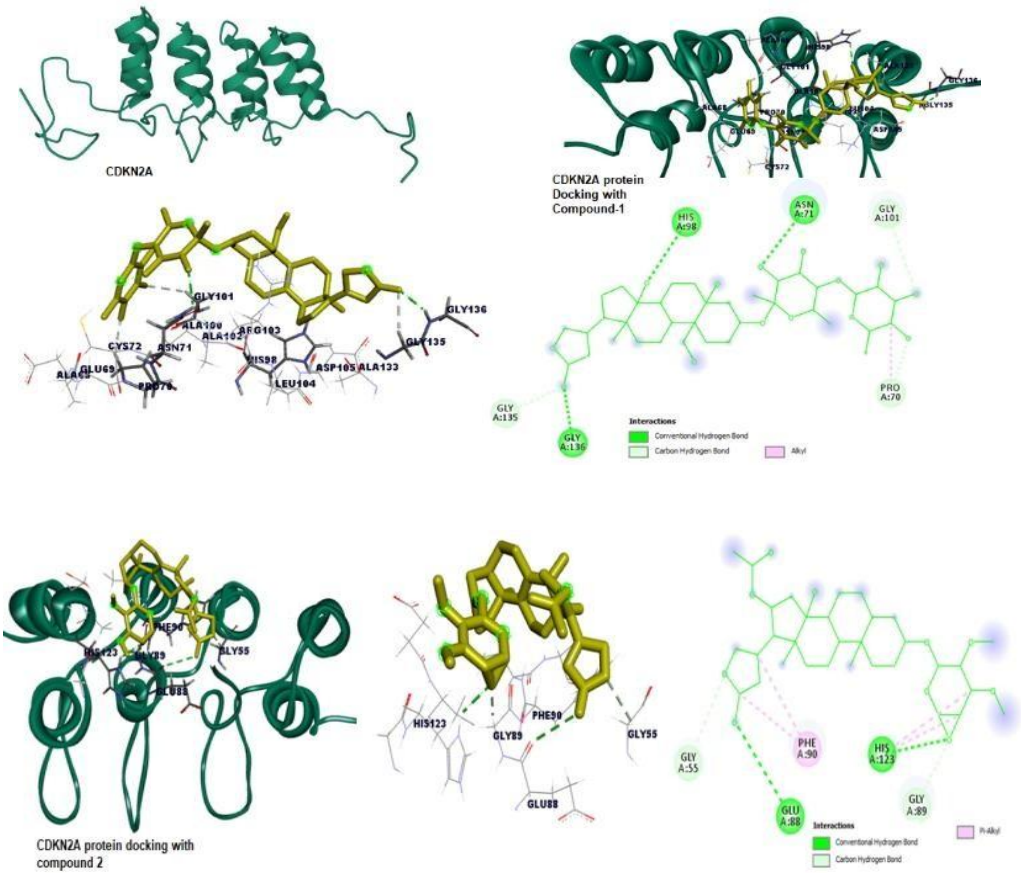


FIG 14:- MOLECULAR DOCKING OF STRUCTURE 9 AND 10 FOR ANTI CANCER ACTIVITY.

Ligands	**H Bonds**	**Bond Energy**	**Binding interactions**
Structure1	3	-7.7	Asn71:HD22 à UNK18:O His98:HE2 à UNK43:O Gly136:HN à UNK42:O
Structure2	2	-5.8	His123:HD1 à UNK36:O UNK31:O à Glu88:O

For anticancer activity when structure 1 and 2 was processed for determining the activity both structure exhibited efficient acivity.

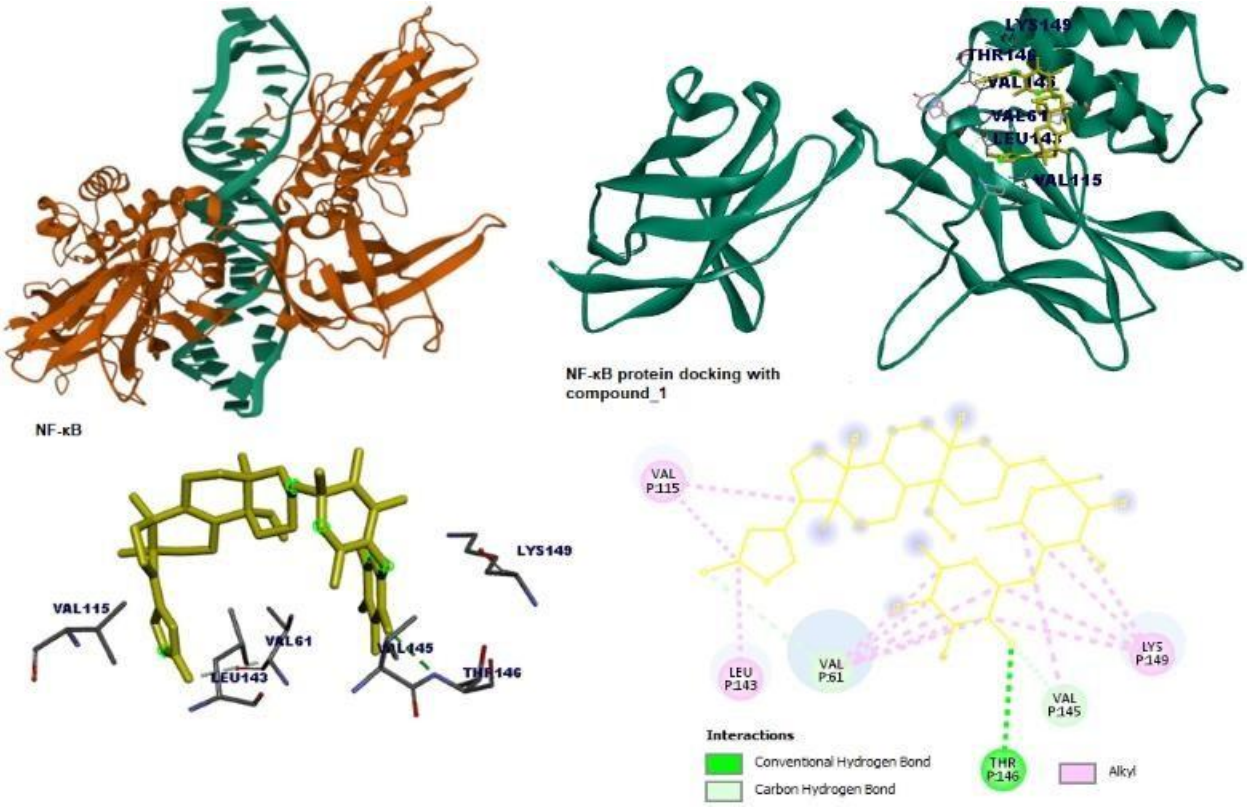


FIG 15:- MOLECULAR DOCKING OF STRUCTURE 9 AND 10 FOR ANTIOXIDANT ACTIVITY.

Ligands	**H Bonds**	**Bond Energy**	**Binding interactions**
Structure1	1	-7.7	Thr146: N à UNK8: O
Structure2	2	-5.9	Ser113: OG à UNK36:O UNK38:O à Asp121:OD1

As initially it was stated that all oxidative stress results in release of radical species which result in causing cancer therefore the selected drug was processed for determination of pharmacological actions by the help of docking studies. The selected drug exhibited action when docked with two different protein with similar activity. The molecule which possess higher negativity of bond energy and no of hydrogen bonds increases result in increase in activity as higher action. Therefore the selected structures where resulted as good anticancer and good antioxidant agent.

The structure 10 the no of hydrogen bonds present for interaction is 3,which interacts and bond energy -7.7 posses higher activity than compare to structure 11 where it as less bond energy and no of site for interaction is less. For anticancer activity.

In the structure 10 the no of hydrogen bonds present for interaction is 1, which interacts and bond energy -7.7 posses lesser activity than compare to structure 11 where it has got less bond energy but no of site for interaction is more than compare to structure 10 therefore can be concluded that structure 11 as good antioxidant activity than structure 10.

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