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"PHYTOPHARMACOLOGICAL EVALUATION OF PHYLLANTUS NIRURI FOR HEPATOPROTECTIVE ACTIVITY"

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Abstract:

For such a lengthy moment, such usage of therapeutic natural compounds for ailment remedy may be dated directly around five centuries to recorded archives of India's ancient humans. Trees has proved to be a significant source of raw compounds for sustaining population wellbeing, especially in the last decade, as more effective methods on herbal treatments are being conducted. The creation of efficient diagnostic medicines relies heavily on medicinal herbs. The condition of the people sickness has traditionally relied on natural materials made from plants, animals, and elements. The development of hepatoprotective, hepatoprotective drugs from traditional medicines has played a key role in the prevention of hepatitis. Over the last half-century, the majority of innovative therapeutic trials of biologically active compounds & associated analogues were focused on hepatic protection. Methanol was used to extract the Phyllanthus niruri flower. Additional phytochemical assays were performed, including the extraction of tannin, alkaloids, glycoside, Column chromatography, preparative TLC, and TLC were used to separate the extract. Sample and substance were subjected to spectral analysis. Pharmacological testing, such as heptoprotective (MTT assay), was also carried out. The identity and purity of herbal extract were determined using standardization parameters, TLC, and Column Chromatography profiles. The application of chromatographic and spectroscopic techniques (NMR, IR, LC-MS) in the isolation and accurate identification of Saponine, alkaloid, and glycoside has been demonstrated. The separated chemical's architecture could be the same as the drug molecule, phyllanthin. Plant chemicals' effects on hepatic conservation was measured in terms of percentage cytotoxicity.

Keywords:

Phyllanthus niruri, MTT Assay, Hepatoprotective

Introduction:

Plant species referenced in ancient writings of Ayurveda and other Indian systems of medicine may be investigated using modern scientific methods to provide improved healthcare leads. Herbal medications and medicinal plants, as well as their extracts and isolated components, have a diverse range of biological activities and have long been used as a food supplement to cure a number of diseases in traditional medicine. Ethno pharmacological research on such medicinally significant plants continues to pique the curiosity of researchers all around the world the numerous undiscovered therapeutic properties that can be investigated in this technique. The antitumor activity of the plant extract as well as the plant's immune modulatory capabilities were investigated. Herbal remedies are in high demand right now, and their popularity is growing by the day.

There are around 500+ medicinal herbs have been referenced. Roughly 1,200 botanicals will be used in tribal therapy approaches, according to historical texts. Roughly 1,200 flora were used in tribal therapy techniques, according to historical documents. The creation along with effective therapeutic agents relies heavily on medicinal plants. Approximately 100 plant-based novel medications were released into the US drug market between 1950 and 1970, including deserpidine and reseinnamie, they're also taken via photosynthetic organisms. Schizophrenia, dermatitis, tuberculosis, insulin, diarrhea, pressure, and carcinoma are all common ailments. are all treated with plant-derived medications. Natural compounds derived from medicinal plants have proven to be effective in cancer treatment. Paclitaxel and camptothein, two natural medicines produced from plants, are thought to account for approximately a third of the global anticancer market.

The usage of as just a provider of phytochemical constituents of healing from affliction may are being dated directly five decades to India's first civilizations' documented records. Trees had also considered to be a great origin of natural element for sustaining human health, particularly for a long in the last decade, as more intense research on natural medicines has been conducted. Not just as medications, and moreover as one-of-a-kind frameworks which could be used as a preliminary step besides synthesized equivalents as well as as a useful tool for good perspective biochemical reactions the herbal species sows a more untapped resource of therapeutically important chemicals Plant chemicals are increasingly being used for medicinal applications in India. Medicinal plants, a primary guide, based on the current Medical Community, would indeed be a range of pharmaceuticals. Traditional medicine, which contains substances derived from medicinal plants, is used by almost 80% of people in developed countries. Products have been studied to become a very useful method of clinically relevant anticancer compounds. However, in the past, ethno pharmacologic data has been underutilized in the hunt for new cancer-fighting principles. Reports of particular anticancer uses of plants are rare in many ethno medical systems, owing to the fact that cancer is a disease with a complicated range of indications and symptoms. Since numerous plant products and their derivatives have been licensed for cancer prevention, new medications that can play a significant role in cancer prevention are desperately needed. A variety of studies have been carried out can

learn more about pharmacological an task Phyllanthus niruri L, and as a result, there are a large number of papers that discuss its pharmacological properties.

Despite the fact that this plant contains a variety of active compounds, questions remain about the active's character chemicals important to sow main therapeutic effect, as well as a processes which they exert their therapeutic effects Consequently, the survey's key point shows an investigation to Phyllanthus niruri L in order to find newly pharmacological possibilities for an variety to diseases.

It will be performed by first locating the components that are the efficient with a specific genetic impact and then determining an effective chemical there was one in charge performance. This effort include a detailed overview of active compounds discovered as a result of Phyllanthus niruri L's likely mechanism of action. This will improve the information base for the creation of novel medications derived from this plant in the future.

The current trend of biologically active agent discovery is associated with herbal plants. Phyllanthus niruri, officially known as Phyllanthus amarus Schum and Thonn, is a plant that grows in the Phyllanthus family. Malaysia, Dukunganak and in Nigeria as IyinOlobe. It belongs to the Euphobiaceae family. Phyllanthus niruri (P. niruri) [1] is one of the widely spread plant all over the Asian regions and expresses several potent biological activities [1]. P. niruri has been used for treatment of diseases and disorders like diabetes, liver disorder Sidney disorders, hepatitis B. Many countries have utilized the aerial section of Phyllanthus niruri in traditional medicine to cure a variety of ailments, including increased libido and male fertility. Indians additionally utilized herb. Treat bradycardia, diarrhea, paroxysmal coughing, itching, arthritic, bronchitis, puffiness, ulcerations, and man genital deficiency. Renal kidney stones are treated with Phyllanthus niruri beverage in Brazil. Is always utilized throughout herbal remedies. In South Africa for hemolytic anemia. Ways for extracting water. Of Phyllanthus aramus Among Nigerians households, it is used to eliminate unused matter and toxins. This shrub easily use in hepatitis, increase blood fluid also modify body defense system this work is associated to develop new pharmaceutical moiety for hepatic treatment.

Experimental:

Extraction of leaf powder:

Phyllanthus niruri leaf powder was made by air drying and pulverizing the leaves. About 25 gramme of crushed leaf content is weighed, 1/5 litter of solvent was added, and this proceed was carried out separately in a Soxhlet apparatus for 7 days at 40-50 degrees Celsius. Until the extract was concentrated, around 40°C, this filtered were dried at room temperature. In a room temperature. After that, this procedure is continued until the sufficient quantity of extracts was achieved. Around 4 degrees Celsius, a purified herb solution was stored until it was needed.

Phytochemical Analysis:

The leaf extracts were freshly prepared and divided into glass jars. Where it was analyzed for various chemical contents using the methods specified. The different Tannins, saponins, carbohydrates, derivatives, alkaloid, terpenoids, and polyphenols were among the synthetic chemicals studied.

***In-vitro* Activity: MTT Assay:**

1) In nutrient broth, Dimethyl sulfoxid (0.2 percent in PBS) and the cell line were maintained. Triplicates of each batch were incubated. To evaluate controlled cellular proliferation and the proportion of viable cells following culturing, standards were kept.

2) Cell cultures were cultured in a CO2 incubator for 24 hours at 37°C and 5% CO2 (Thermo scientific BB150)

3) During incubating, the solution was withdrawn entirely then 20 l o is introduced.

4) Tubes were seeded in a CO2incubator for 4 hours once MTT was added.

5) Examined the a well under a magnification for the growth of ferrous ion. Only live cells changed the yellowish MTT to a dark coloured azo dye. After entirely clearing the material (Reg. No. - 18313003120315668).

6) Added 200µlof DMSO (kept for 10 min) and incubate at 370C (wrapped with aluminium foil).

7) Triplicate samples were analysed by measuring the absorbance of each sample by a microplate reader (Benesphera E21) at a wavelength of 550 nm.

Isolation of active constituents:

Column Packing -

Chloroform was poured into a 20-centimeter column. Silica gel (mesh size 60-120) was dissolved in petroleum ether and put into the column as slurry. The excess solvent was drained off during the setting phase, and an extra quantity of the slurry was added. This technique was repeated until the column reached 45cm in height. To minimize disruption of the packed silica gel during the addition of solvent, a circle of filter paper was placed over the surface of the silica gel and a little amount of acid washed sand was placed over the filter paper.

Elution

The dried extract of *Phyllanthus niruri* leaves (5 g) was ground with a little amount of silica gel and was made into slurry. This powdered slurry was loaded on to the column. Chloroform and methanol was along with eluents. An sample extract flow rate was changed to 0.5ml per 2min throughout that operation. Pure petroleum ether was used for elution followed by the addition of ethyl acetate in the ratio of increasing concentration of 1%, 2%, 3%. Separate portions (20 ml) was taken then TLC evaluated to pure compound. Those portions which exactly same as was combined plus condensed.

Chromatographic Analysis:

Partially purified crude Ethanolic extract was loaded on to TLC and It became realized by means of an organic solvent with in proportion of 2:2:6 (Butanol: acetic acid: water) A single non-visible area is glowing and emits Ultraviolet light at 360nanometers.

Preparative TLC:

In preparative TLC glass plate size 20 multiply 20 is used upon the late silica gel –g is poured and uniform thickness is coved on the plate .then the plate is activated to 100 degree in microwave oven. Then reedy to use the plant extract was put to a plate and developed in a 3.1:4.1:0.5 N-HEXAN, ETHYL ACETATE AND FORMIC ACID .solvent solution (mobile phase). The chromatogram plate spots were air dried and examined in an iodine chamber. The Rf values were calculated and determined to be 0.8, which is similar to Quercetin.

Spectroscopic Analysis:

Ultra violet results are taken from UV Shimadzu, UV-1800, Japan visible spectrophotometer. IR spectra were measured on a ATR-Bruker spectrometer. Silica gel was used for column chromatography (60-120 mesh).HRMS data were obtained on an LC-QTOF MS/MS mass spectrometer series 6540 instrument. NMR spectra were recorded on PicoSpin 80 NMR spectrometers.

Results and Discussion:

Phytochemical Screening:.

Extrct	steroids	Tri-terpenoids	alkaloid	flavonide	saponine	tanin	Giyco-sides	lignan	Volatile
Diethyl Ether	+ve -ve	-ve -ve	+ve +VE	+ve +ve	-	+ve	+ve	-	+
chloroform	-ve -ve	+ve -ve	-ve +ve	-ve -ve	-	+ve	+Ve	-	+
Ethyl Acetate	+ve +ve	-ve +ve	+ve +ve	-ve +ve	-	-ve	+VE	-	-
Acetone	-ve +ve	-ve -ve	-ve -ve	+ve +ve	-	-ve	-Ve	-	+
methanol	+ve +ve	+ve -ve	+ve -ve	-ve -ve	-	+ve	+ve	+	-
Hydro-alcoholic	+ve +ve	-ve -ve	+ve -ve	-ve -ve	-	-ve	+ve	-ve	-ve
Pet-ether	-ve	+ve	-ve	-ve	-	-ve	+ve	+ve	+ve

PARTIAL CHARECTERIZATION OF PHYTOCONSTITUTION FROM THE LEAVES OF PHYLLANTHUS NIRURI BY **COLUMN CHROMATOGRAPHY.**

ACTONE: METHANOL 80-20

ACTONE: METHANOL 60-40

ACTONE: METHANOL 40-60

ACTONE: METHANOL 20-80

METHANOL 100

Figure. 5. Column Chromatography of methanol extract

Of phyllanthus niruri

Chemical portions then separated via TLC with acetone: methanol. On recurrent purifying using methanol, the large band-6, and acetone: methanol (50:50), yields the colorful chemical phyllanthin. Shendo's test, glycosides test, and Molisch test are all passed by the purified substance.

PARTIAL CHARECTERIZATION OF PHYTOCONSTITUTION FROM THE LEAVES OF PHYLLANTHUS NIRURI BY PTLC

For chromatographic thin layer chromatography, glass plates (20 x 20 cm) were liberally coated (0.4-0.5 nm) with silica gel 'G' (45 gm/80 ml water), activated at 100°C for 30 minutes, and cooled at room temperature

(PTLC). The plant extract was put to a plate and produced N-HAXANE, ETHYAL ACETATE, and FORMIC ACIDE in the ratios of 3.1:1.4:0.5. The chromatogram plate spots were air dried and examined in an iodine chamber. The Rf values were calculated and determined to be 0.8, which is similar to phyllanthin.

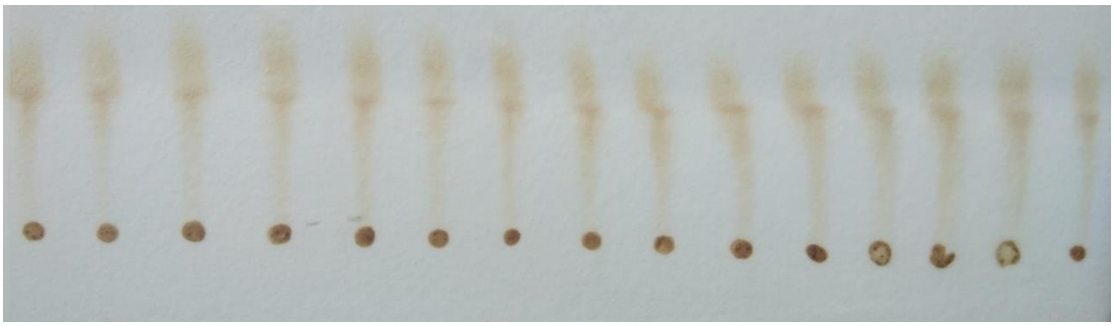
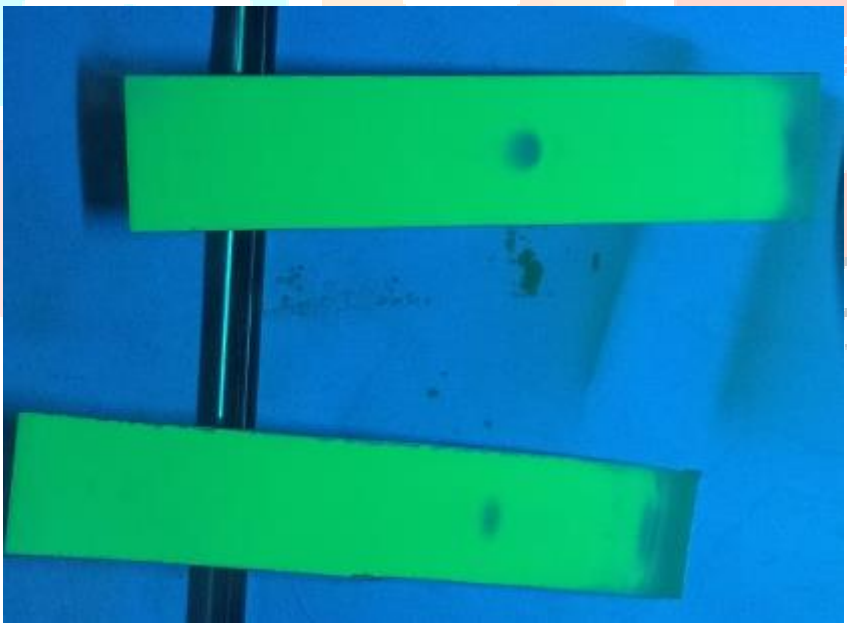


Figure. 7. Preparative Thin Layer Chromatography ethanol of phyllanthus niruri



Rf value– 0.8
Stationary phase – Silica gel,
Mobile phase – N-HAXANE, ETHYAL ACETATE, FORMIC ACIDE. 3.1:1.4:0.5. V/v/v

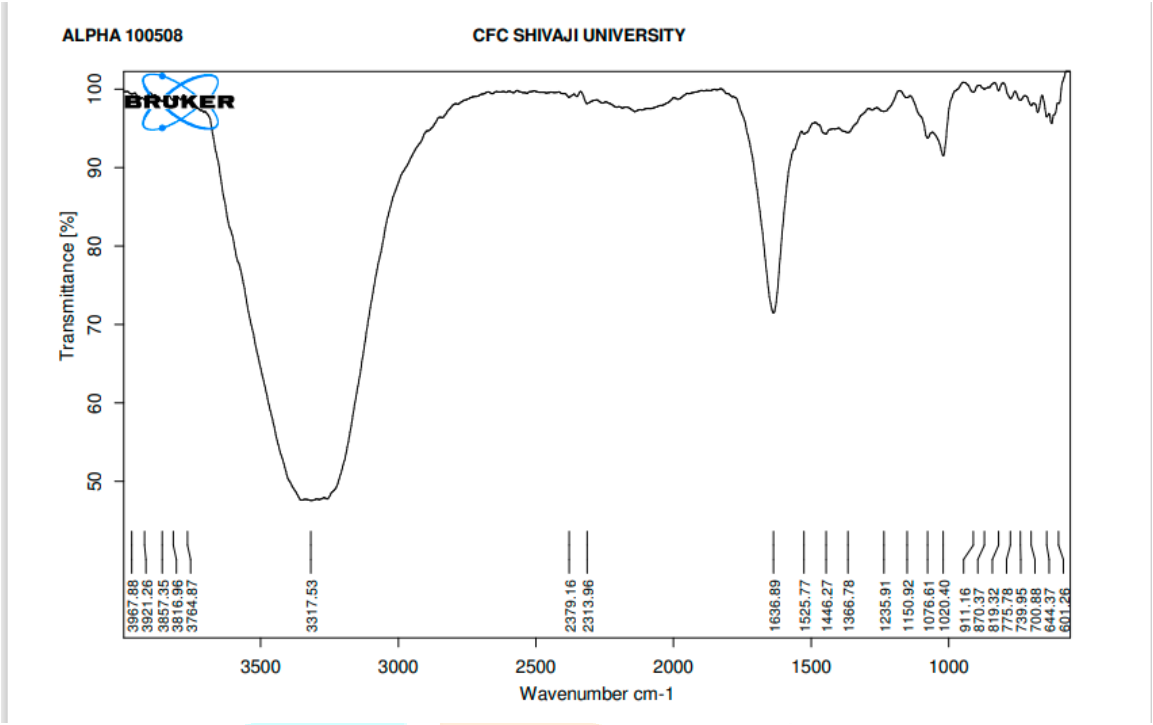
SR NO	CONCENTRATION	RF VALUE
1	80-20	0.6
2	60-40	0.7
3	40-60	0.6
4	20-80	0.6
5	100	0.5

-IR.

INTERPTATION-

IR Absorptions of Common Functional Groups

Functional Group	*Absorption Location (cm^{-1})*	*Absorption Intensity*
Alkane (C–H)	2,850–2,975	Medium to strong
Alcohol (O–H)	3,400–3,700	Strong, broad
Alkene (C=C)	1,640–1,680	Weak to medium
(C=C–H)	3,020–3,100	Medium
Alkyne		
(C≡C)	2,100–2,250	Medium
(C≡C–H)	3,300	Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N–H)	3,300–3,350	Medium
Carbonyls (C=O)		Strong
Aldehyde (CHO)	1,720–1,740	
Ketone (RCOR)	1,715	
Ester (RCOOR)	1,735–1,750	
Acid (RCOOH)	1,700–1,725	

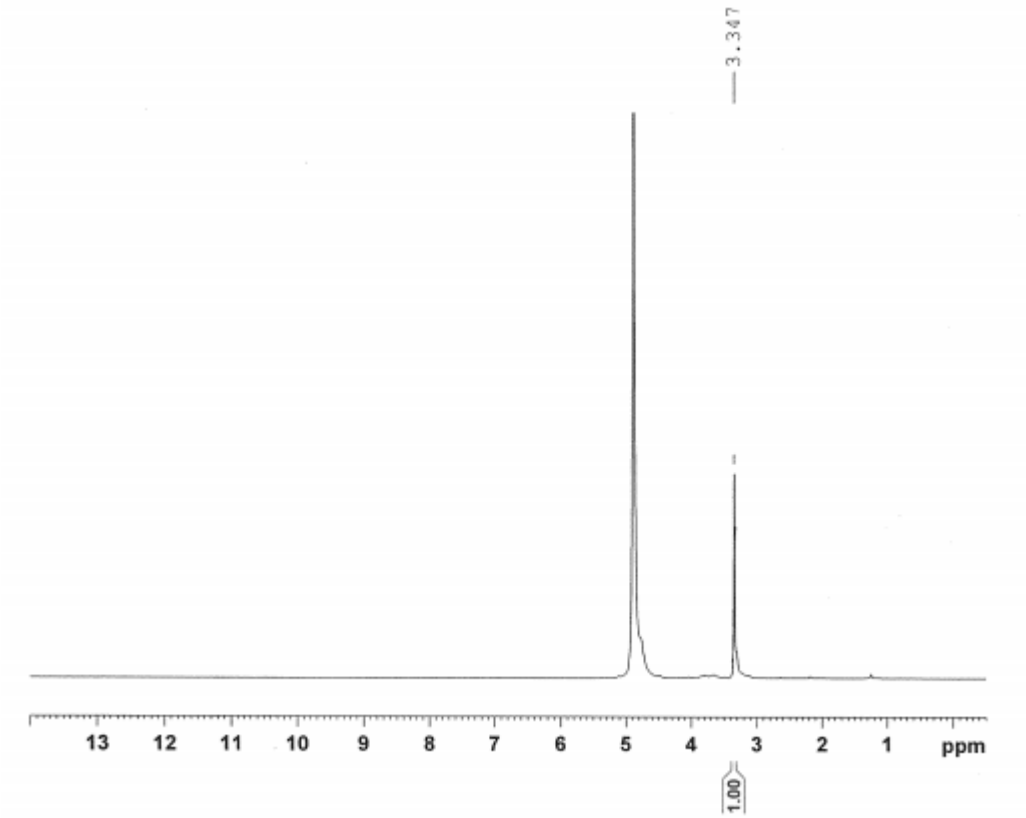


Functional Groups	Standard Frequencies (cm-1)	Observed Frequencies (cm-1)
OH. carbohydrate.protine,pheno	3500-3000	3305.65
C=C,C=C	2500-2000	2378, 2313
AROMATIC	2000-1500	1982.22
C=C ALKENE	2000-1500	1637.85
C=O INORGANIC COMPOUND	1400-1460	1452.09
1350-1440	CH ,CH2 ALIPHATIC BENDING	1366.19
1200-1300	CN,AMIDE BOND	1229

-NMR

Table 12-NMR Interpretation.

TYPE OF HYDROGEN	CHEMICAL SHIFT
(CH3) (TMS)	0
R2NH2	0.5-5.0ppm
ROH	0.5-6.0ppm
RCH3	2.3-1.0ppm
RCH2R	1.2-1.4
R3CH	1.4-1.7
R2C=CROHR	1.6-2.6
CH=CH	2.O-3.0
R-C=O-CH3	2.1-2.3
R-C=OCH2R	2.2-2.6
AR-CH3	2.2-2.5
CH2R	2.3-2.8
RCH2OR	3.3-4.0
RCH20H	3.4-4.0
R-C=O-COCH3	3.7-3.9
R-C=O-OCH2R	4.1-4.7
AROH	4.5-4.7
R-C=O-H	9.5-10.1
R-C=O-OH	10-13



Sr No	**Functional Group**	**Observed Frequencies**
1	Aromatic alcohol	5.3
2	ester	4.8

-MASS.

MASS -

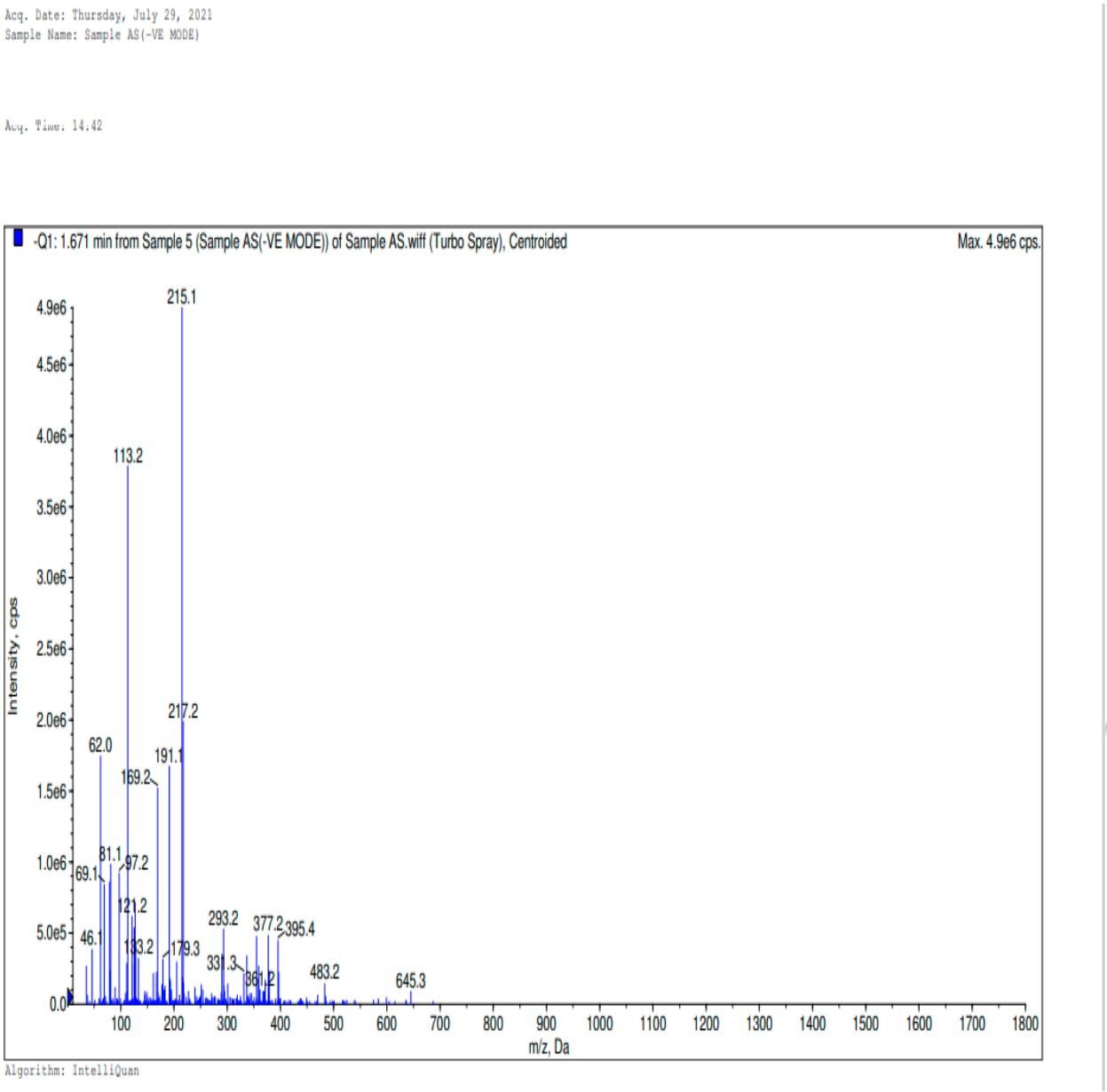


Figure 11 -mass spectra

MASS INTERPRETATION.

Table no -13

MOL.WT	CHEMICAL NAME.
215	Astatine and his isotopes.
113.2	Benzene. Chlorine anion.
117.2	Pyrimidine. 5amino -4.6dichloro -2 ethyl.
169.2	Adenine 2chloro. 4-fluro-2 nitrobenzaldehyde. Ethyl dinitroacetobenzaldehyde. 1-nitro-4-(methymercaptobenzene) Thiazole.
62.01	H2N-NO2 C5D DC5AION Boric acid. Dimethyl sulfide +ve ion AL2D4
121.2	DL-CYSTEINE. L-CYSTEINE.
645.4	Galactose, fructose, malose.
395	(4-fluro-3-nitrophenyl)methanol (dimethylpentaflurophynylsilyl ether)
293.2	o-methyl(dimethyl glyoxime)copper2.dinitroethoxy)-1.3.5triazine.

Conclusion:

MTT Assay was used to explore the significant hepatoprotective properties (Saponine, alkaloid, and glycoside) of phyllanthus niruri in vitro. The tannin, alkaloid, and glycoside chemicals employed in the experiments are abundant in the leaf of phyllanthus niruri. A phytochemical examination of leaves extractive from the herbs tested revealed the presence of flavonoids, tannin, glycosides, and terpenoids, in addition to terpenoids. Phyllanthus niruri anticancer substances showed hepatoprotective efficacy in the MTT assay. 1H, NMR infrared spectroscopy, and mass spectrum analyses were used to describe the flavonoid molecule.

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