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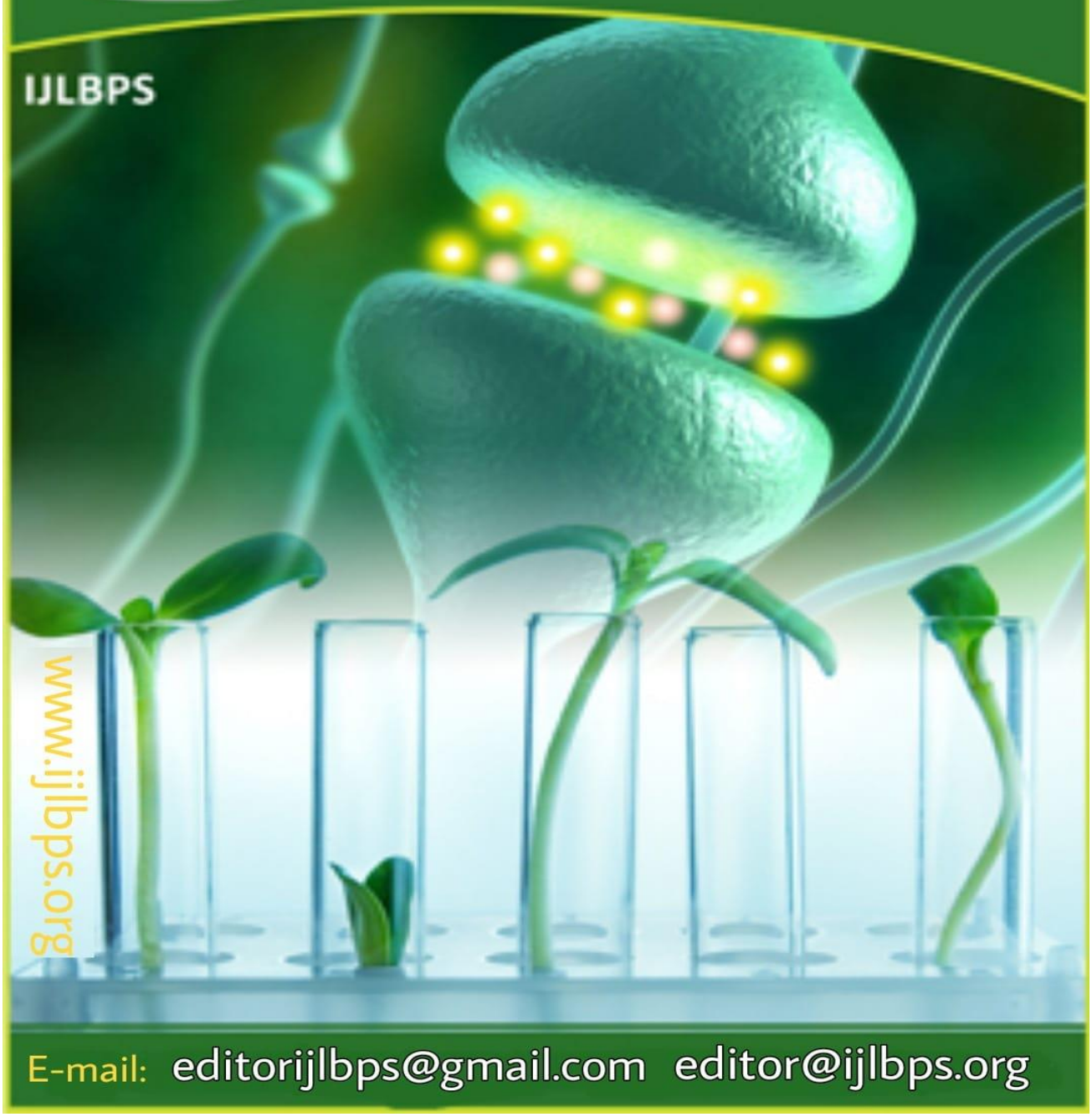

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The fruit of the plant Terminalia belerica Roxb. : a phytochemical and pharmacological assessment

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Abstract

India has a wealth of documented and widely used information on herbal medicine. Only a small handful of the world's most valuable medicinal plants are not native to the United States. There are about 3,000 plants with documented therapeutic uses in India. This publication provides a comprehensive review of the phytochemical and pharmacological analysis of Terminalia bellerica. Using IR and GCMS analysis, an attempt was made to separate ellagic acid and describe it.

Terminology: ellagic acid, anticancer action, and Terminalia bellerica

Introduction

Herbal remedies are made from different parts of plants, such as leaves, stems, roots, bark, etc. They are often used for the treatment of minor or chronic conditions, and they include potentially biologically active substances. Approximately 75% of the pharmaceutical requirements of developing nations are met by plants used in traditional, folk, and herbal medicine in India. Warts caused by viruses are quite frequent; the vast majority of individuals will get at least one throughout their lifetimes. The DNA human papillomavirus (HPV) causes warts, and DNA sequence research reveals that there are more than 90 subtypes of HPV. Terminalia bellerica Ro xb. is a huge deciduous tree that may grow up to 12 meters in height. It is a member of the Combertaceae family and can be found in plains and woodlands up to an altitude of 900 meters. About 2.5–4.0 cm in diameter, the fruit is almost spherical to ovoid. The ripe fruits are a wrinkled gray or grayish brown in color, range in thickness from 3 to 5 millimeters, and have an astringent flavor. Anti-diabetes, la xat ive, anti-cancer, and antibacterial properties are some of its many uses. Its primary use is in cosmetics for the hair and skin. It protects the liver and also has antioxidant properties. A survey of the relevant literature suggests that the plants in question have anti-oxidant, anti-inflammatory, anti-pyretic, anti-ulcerogenic, and hepatoprotective properties.1-4

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New, effective, and non-toxic anticancer medicines derived from local plants are the focus of current studies.agents. Their chemical make-up, mechanism of action, and toxicology have not yet been determined. The current investigation focuses on a pharmacological and phytochemical analysis of the fruits of Terminalia belerica Roxb. (Combretaceae), with a focus on their anticancer effects in experimental animals. Herbal remedies are often safe and effective, with no negative effects, and they are inexpensive medications that will help the people of these countries. The plant was chosen with this in mind.

Material and Methods

The fruits of the plant component were taken in the month of July 2009 from the Botanical Garden Pachmadhi in the district of Hoshangabad, Maharashtra, India, and were afterwards taxonomically identified by Dr. B. Dubey, Head of the Botany Department at the Government Girls' Postgraduate College in the nearby town of Moti Tabela in the Indore district of Maharashtra. The plant materials were air dried in the shade for ten to fifteen days. Mechanical mixer grinding was used to accomplish the grinding process after the plant material was dried. Terminalia belerica fruit powder was defatted using a cold maceration procedure with petroleum ether at room temperature. Aqueous Methanol (50%) extract was generated by a cold maceration method from the defatted powder material (mark) thus obtained. The solvent was evaporated away in a low-pressure distillation process, and the resultant semisolid material was dried in a hot air oven and water bath.

Pharmaceutical Research The Fight Against Cancer

When a chemical causes harm shortly after being applied topically, it is said to have an acute cutaneous toxicity. The investigation was conducted to find out how much of the Aq. methanolic e xtract might be safely consumed. The extracts' acute cutaneous toxicity was evaluated by topical application of the strongest extracts. No. 402 of the OECD Guidelines (OECD Guidelines, 1987) served as the basis for the research. The institute's animal ethics committee 1283C 09 CPCSEA reviewed and approved the experimental procedures. The toxic ity investigation included five female rats. A single dermal application of 2000mg/kg body weight of extract was monitored for 14 days.

The animal population was evenly split into seven sections. There are six creatures in each set. After first shaving a 2 cm2 patch of skin in the interscapular region using hair removal lotion, mice had their hair trimmed with scissors every 2 weeks. Shaved areas received topical treatments according to the following procedure for up to 16 weeks.

Division 1: (Normal, untreated subjects) Currently, there is no therapy. 100 ml acetone twice weekly for 16 weeks (control group), Third group (DMBA only) DMBA (104 g) was diluted in acetone (100 l) and applied topically once. In Group 4, DMBA was used, and then 1% croton oil was given to the skin twice weekly for 16 weeks. In Group 5, 100 l of DMBA was administered an hour before each application of 1% croton oil and Terminalia belerica Ro xb. extract was applied. Croton oil alone (Group 6) and Terminalia belerica Roxb. extract alone (Group 7)

Separation of Effective Ingredients Chromatography, Column

A little amount of glass wool was used to seal the column's base before a fresh sand bed was added. The sand foundation ensures a level surface for the adsorbent column. The column was then loaded with dried Silica Ge l 100-200 mesh. When the powder reached about two-thirds of the column, it was tabbed and put away. To prevent the adsorbent from being disturbed when developing stages of new mobile phase were introduced to the column, a filter paper disc and sand bed were put over the adsorbent. We employed a filter paper disk to separate the active components from an aqueous methanol (50%) extract of Terminalia belerica Roxb. Column chromatography over silica gel 100-200 mesh was used to refine the crude Aqueous methanol extract of Terminalia belerica Roxb. Solvents of increasing polarity were used to elute the column. Each fraction was captured in a 25 ml vial and evaporated to provide the final product. Thin-layer chromatography (TLC) was

used to analyze each fraction for the presence of certain components.

Thin Layer Chromatography
- Silica gel G was wet in required quantity.
- Homogenous slurry was made with sufficient water.
- Then the slurry was poured into TLC glass plate by spreading technique and uniform silica gel layer was adjusted to 0.25 mm thickness.
- The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-150^{0}C for 1hour and then used for TLC.
- The extracts were prepared with respective solvents like methanol, ethanol and distilled water and made upto 10 ml indifferent test tubes.
- Then with the help of capillary tube extracts was spotted on TLC plates, which were developed in TLCchamber, previously saturated with different solvent systems.
- By trial & error method, Aq. methanolic extracts showed isolation and dissolution with following solventsystem.
  - Ethyl acetate : Toluene : Methanol : Glacial acetic acid :(7.5 : 2 : 0.5 : 0.2)
- The different spots developed in each solvent system, the Rf value were correspondingly calculated.

Characterization of the isolated plant constituents

Thin Layer Chromatography

IR Spectrophotometer (FTIR 8400 SHIMADZU)

GC-MS (IIT Pavai)

Description of the isolated compoundBy TLC

Elution of compound from crude Aqueous Methanol extract

Colour: Yellow sticky amorphous waxy samisolid

Solubility : Water, Alcohol, Acetone

Melting Point : 210^0 C

Rf Value : 0.59 (Ethyl acetate:Toluene:Methanol:Glacial acetic acid)

Spectral studies of isolated compoundBy IR Spectroscopy

Infra-red spectrum of the fraction obtained from the column chromatography of the Aq. Methanolic extract of *Terminalia belerica* Roxb. was investigated for its characteristic functional groups. All the peaks obtained by IR-Spectroscopy are shown in figure no

Instrument used : Perkin Elmer FT-IR (Shimadzu)

Method: Neat spectra Wave Number : 4000 – 450cm^{-1}

By GC-MS Spectroscopy

GC-MS spectrum of the fraction obtained from the column chromatography of the aqueous Methanolic extract of the *Terminalia belerica* Roxb. was investigated for its molecular weight to characterized the fraction by its massand mass of fragments, all the peaks obtained by GCMS spectroscopy are shown in figure.

Results and Discussion

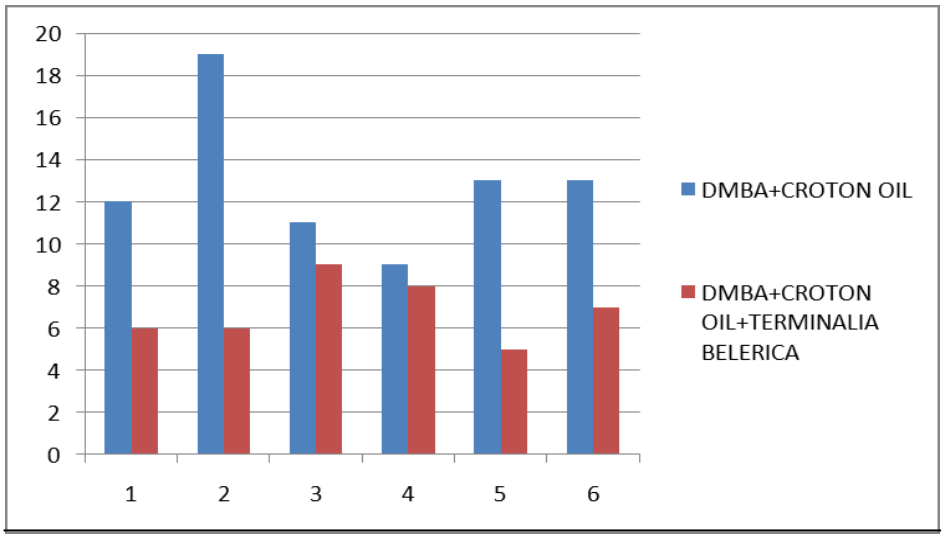
Based on literature, the plant part was collected from Botanical Garden Pachmadhi, Distt. Hoshangabad, M. P, and been authenticate. The dried plant part was subjected to extraction sing different solvents i.e., petroleum ether, acetone: methanol, and aqueous methanol. The results of extractive values are given in table no. 1. Then the Preliminary Phytochemical screening was carried out in which the active compounds were mentioned (table 2) and the ash values were mentioned (table 3).The dermal acute toxicity was studied and present in (table 4). It was found that the limit dose is 2000 kg/body weight. The effect of aq. methanolic extract on DMBA induced papilloma in Swiss albino mice was carried out. The results are shown in (table 5). This was found to be significant. The Aq. Methanolic extract was subject to column chromatography (table 7) and was identified by TLC, by which the R_f value was found to be 0.59. The isolated compound was confirmed by IR, GC and MS (shown in table 9 and figure 5, 6 and 7). The isolated compound was 2, 3, 7, 8 - Tetraoxy-chromeno[5,4,3-cde]chromene-5,10-dione, having molecular formula $C_{14}H_2O_8$ and molecular weight is 298.19 g/mol

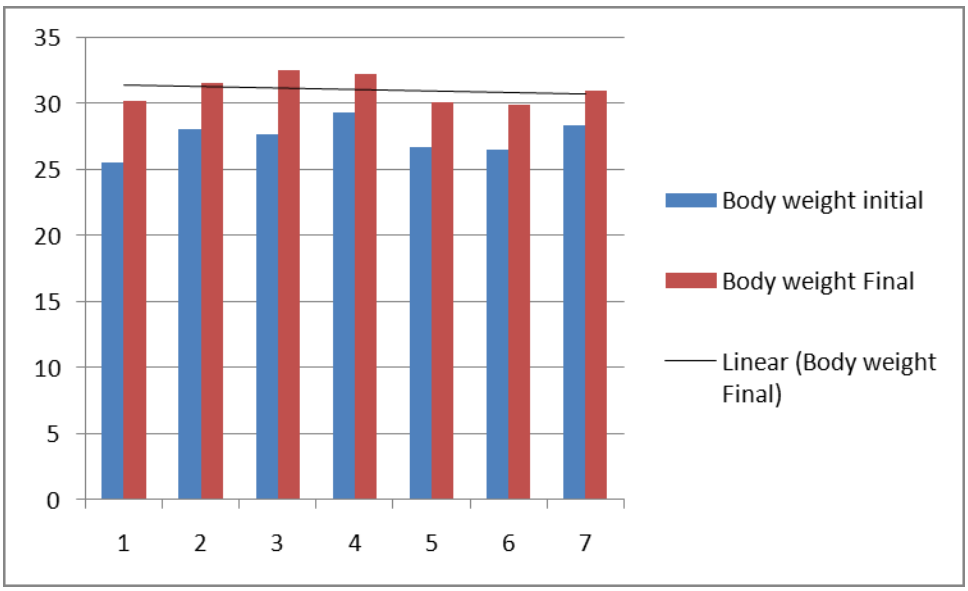
.

Table No. 1 Effect of *Terminalia belerica* Roxb. On DMBA-induced papillomas in S wiss albino mice

Groups	Body weight		No. of Papilloma (Avg.)	Tumour
	Initial	Final		Yield
No Treatment	25.48	30.15	---No---	--
Vehicle alone	28.04	31.56	---No---	--
DMBA Alone	27.60	32.48	---No---	--
Croton oil alone	29.30	32.15	---No---	--
DMBA+ Croton	26.65	30.30	13	1.8
DMBA+ *Terminalia belerica* Roxb. + Croton oil	26.50	29.85	6	1.1
Terminalia belerica Roxb. Alone	28.30	30.95	---No---	--

Figure No. 1 The number of Papilloma found in Individual mice





igure No. 2 Effect on body weight by development of papilloma cancer

No. of fractions	Eluent	Ratio	Colour of the fraction	Rf values
F1 – F7	Petroleum ether	100	No	-
F8 – F14	Pet. Ether : Chloroform	90:10	No	-
F15 – F18	Pet. Ether : Chloroform	60:40	Greenish	0.89
F18 – F20	Pet. Ether : Chloroform	50:50	No	-
F21 – F23	Chloroform	100	No	-
F24 - F27	Chloroform : Ethyl acetate	90:10	Reddish	0.64

table No. 2 Details of column chromatography fractions of Aq. Methanolic extract of Fruit of *Terminalia belerica* Roxb.

F28 – F30	Chloroform : Ethyl acetate	60:40	No	-
F31 – F33	Chloroform : Ethyl acetate	50:50	No	-
F34 – F37	Chloroform : Ethyl acetate	10:90	No	-
F38 – F41	Ethyl acetate	100	No	-
F42 – F45	Ethyl acetate : Ethanol	90:10	Yellowish	0.56

Table No. 3 TLC of Aq. Methanolic extract of Fruit of *Terminalia belerica* Roxb.

Sample	Solvent system	No. of spots	Colour of spots	R_f Values
Acetone: Methanol extract of *Terminalia belerica*	Chloroform : Acetic Acid : Water (5 : 4.5 : 0.5)	4	Yellowish Pink	0.75
D. Water Methanol extract of *Terminalia belerica*	Ethyl acetate:Toluene: Methanol: Glacial Acetic Acid (7.5 : 2 : 0.5 : 0.2)	1	Pinkish Light Yellow	0.59

Figure No. 3 IR Spectra of *Terminalia belerica* Roxb.

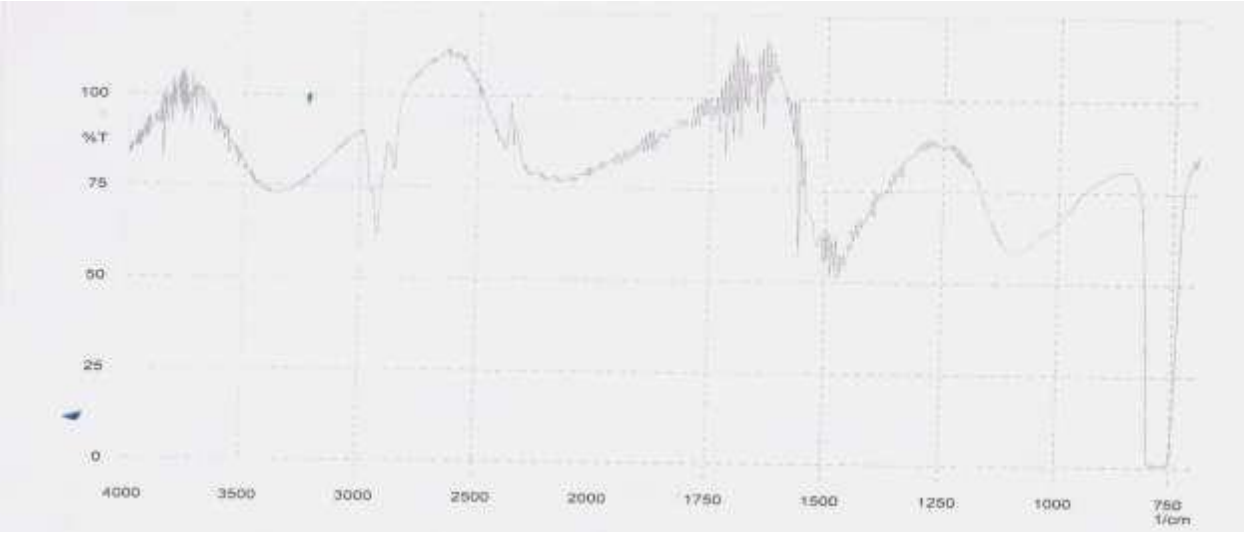


Table No. 4 IR – Data of Isolated Compound

Wave number cm $^{-1}$	Comments
3441	-OH Bonded, Broad.
2908	C-H Stretching
2849	C-H Stretching
1598	COO^- Stretching, Asymmetric.
1467	COO^- Stretching, Symmetric.
1142	C-O Stretching, Acyclic.

Figure No. 4 Gas Chromatography s pectra of isolated compound

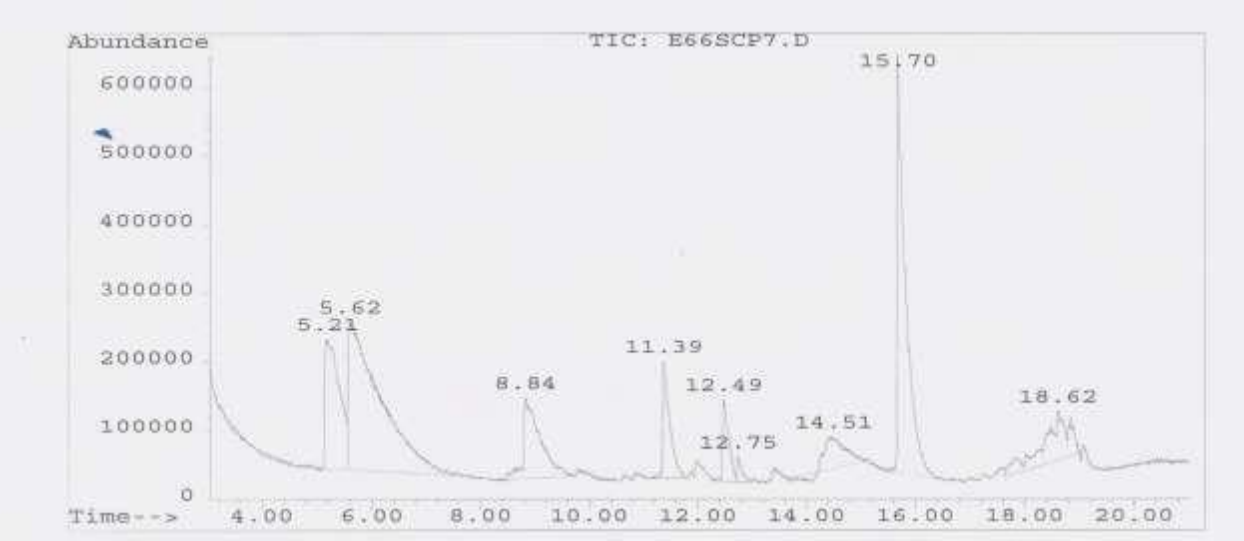
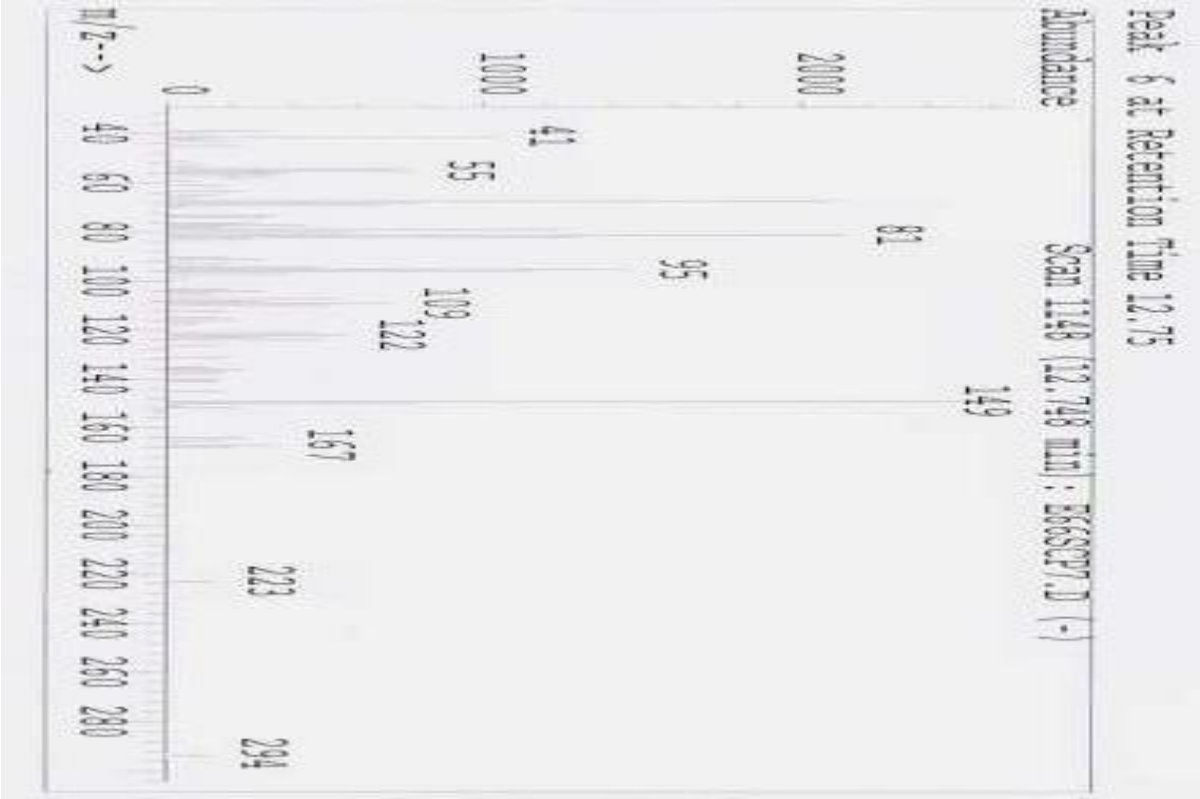
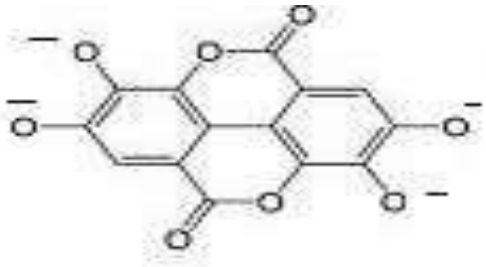


Figure No. 5 Mass Spectrum of isolated compound



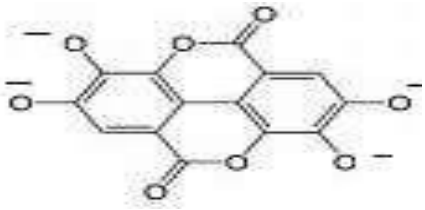
Structure of Isolated Compound



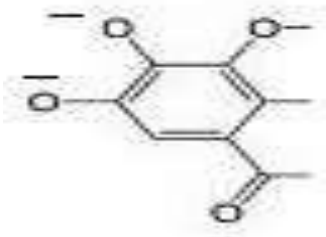
2, 3, 7, 8-Tetraoxy-chromeno[5,4,3-cde]chromene-5,10-dione

Molecular Formula : $C_{14}H_2O_8$
Molecular weight **:** 298.19 g/mol
IUPAC name **:** 2, 3, 7, 8-Tetrao xy-chro meno[5,4,3-cde]chromene-5,10-dione

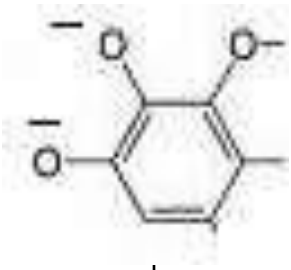
Fragmentation of mass spectra of isolated compound:



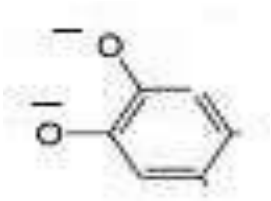
M/z=298 -M/z=149



M/z=149 -M/z=29



M/z = 29 M/z = 123



M/z=93

Synopsis and Final Thoughts

The plant was chosen after considering its historical use and the results of existing research. Terminalia belerica Ro xb. has been studied for its phytochemical and pharmacological properties. In accordance with OECD Guidelines no. 402 (OECD Guidelines, 1987), acute oral toxicity experiments demonstrated that Terminalia belerica Roxb. acetone:methanolic and Aq. Methanolic extracts had no impact at 2000mg/kg. As a result, a dosage of 200 milligrams per kilogram was used. The cancerous papilloma was caused by DMBA. Two times weekly for up to 16 weeks, 100 l of DMBA dissolved in acetone was applied topically. Cancer was induced with DMBA and promoted with 1% Croton oil administered twice weekly for 16 weeks. A total of 100 l of extract was used. Because of its intermediate polarity between Methanol and water, Aqueous Methanol Extracts was chosen. Because of this, effective ingredients were separated.

employing a column chromatograph with liquids of varying polarities. All of the portions went through TLC. This 100% ethyl acetate contains just one of the following ingredients: Rat io (7.5:2) toluene, methanol, glacial acetic acid. The IR and MASS spectra of the pure compound show that its refractive index is 0.59. The compound's structure was deduced from the analysis of the available data. The structure of the isolated compound 2,3,7,8-tetraoxachromeno[5,4,3-cde]chromene-5,10-dione was determined by analyzing its spectrum data. Terminalia belerica Roxb. has shown promise in preliminary phytochemical and pharmacological tests, and further research is needed.

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