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**Phytochemical and pharmacological evaluation of the plant fruit  
of *Terminalia belerica* Roxb.**

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**Abstract**

India is sitting on a gold mine of well-recorded and traditionally well-practised knowledge of herbal medicine. There are very few medicinal herbs of commercial importance which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. The present paper enumerates the phytochemical and pharmacological evaluation of *Terminalia bellerica*. Attempt was made to isolate and characterized ellagic acid on the basis of IR and GCMS studies.

**Key- words:** *Terminalia bellerica*, ellagic acid, anticancer activity

**Introduction**

Herbal medicines are prepared from a variety of plant materials as leaves, stems, roots, bark etc. They usually contain may be biologically active ingredients and are used primarily for treating mild or chronic ailments. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries. Viral warts are extremely common and most people suffer from one or more at some point during their life. Warts are a result of infection with the DNA human papilloma virus (HPV), of which there are over 90 subtypes on the basis of DNA sequence analysis. *Terminalia bellerica* Roxb. belonging to family Combretaceae is a large deciduous tree, 10-12 m or more high, commonly found in Plain and forests up to 900 m elevation, fruits ripen towards November. Fruit nearly spherical to ovoid, 2.5-4.0 cm in diameter. The mature fruits grey or grayish brown with slightly wrinkled appearance, the thickness from 3-5 mm and the taste is astringent. It is used as anti-diabetics, laxative, anticancer, antimicrobial. It's more used in the hair product and skin products. It has antioxidant property and hepatoprotective activity. Literature review reveals that the plants possess antioxidant activity, analgesic, antipyretic and ulcerogenic Effect, hepatoprotective activity and antimicrobial activity.<sup>1-4</sup>

The aim of research is to find out new anticancer drugs from indigenous plants which are potent and nontoxic agents. Their chemical characterization, mode of action and toxicity studies is yet to be established. Present study deals with Phytochemical and pharmacological evaluation of *Terminalia belerica* Roxb. Fruits (Family: Combretaceae) with special reference to, anticancer activities in animal models. Normally herbal products are free from side effects/adverse effect and they are low cost medicines, which will be beneficial for the people of these country. Keeping this in view, the plant was selected.

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## Material and Methods<sup>5-7</sup>

The fruits of the plant part was collected in the month of July 2009 from the Botanical Garden Pachmadhi, Distt. Hoshangabad, M. P., (India) and was taxonomically identified by the botanist Dr. B. Dubey, HOD, Dept. of Botany, Govt. Girls PG College, Moti Tabela, Indore dist. M. P. (India). The plant materials were shade dried at room temperature for 10-15 days. After the drying of plant material, grinding had been done by using the mechanical mixer grinding. The dried powdered fruits of *Terminalia belerica* were defatted with petroleum ether by the cold maceration process at room temperature. The defatted powder material (mark) thus obtained was further extracted with Acetone-Methanol, and Aqueous Methanol (50%) extract was prepared by cold maceration process. The solvent removed by distillation under low pressure and the resulting semisolid mass was dried using water bath and hot air oven at the temperature maintain in between 30 to 45°C.

### Pharmacological Studies

#### Anticancer Studies

Acute dermal toxicity is the adverse effect occurring within a short time of dermal application of a single dose of test substance. The study was carried out to determine the maximum tolerable dose of the Aq. methanolic extract. The acute dermal toxicity testing of the extracts was done by direct applying the extracts of the highest concentrations. The OECD guidelines no. 402 (OECD Guidelines, 1987) were followed for the study. The experimental protocols were subjected of the sensitization of the institute animal ethical committee 1283\C\09\CPCSEA and cleared by same. Five female rats were taken for the toxicity study. Extract of 2000mg/kg body weight in a single dose was applied by dermal route and observed for 14 days.

The animal was randomly divided in to 7 groups. Each group comprises of 6 animals. The skin of mice were shaved in 2 cm area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatments were provided topically on shaved area up to 16 weeks using the following protocol.

Group 1 : (Untreated control) No treatment., Group 2 : (Vehicle control) 100µl acetone 2 times/weeks up to 16 weeks, Group 3 : (DMBA alone) 104µg DMBA was dissolved in 100µl acetone and a single application was given, Group 4 : DMBA + Croton Oil: DMBA followed by 1% Croton oil applied on skin 2 times a weeks up to 16 weeks, Group 5 : DMBA + Croton Oil + Extract of *Terminalia belerica* Roxb., 100µl dose one hour before the each application of 1% croton oil, Group 6 : Croton oil alone and Group 7 : Extract of *Terminalia belerica* Roxb. alone

### Isolation of active constituents

#### Column Chromatography

The bottom of the column was first plugged with little glass wool and then clean sand bed was placed over the glass wool. The sand bed serves to give a flat base to the column of the adsorbent. Then the dried Silica Gel 100-200 mesh was poured into the column. After 2/3<sup>rd</sup> of the column was filled with the powder, it was tabbed, and set aside. After that, a filter paper disc and sand bed were placed over the adsorbent in order to avoid the disturbance of the adsorbent, as fresh mobile phase was added to the column in the initial stages of development. The Aq. methanol (50%) extract of *Terminalia belerica* Roxb. was placed over the filter paper disk and used to isolate the active constituents. The crude Aqueous methanol extract of *Terminalia belerica* Roxb. was subjected to column chromatography over silica gel 100-200 mesh. The column was eluted with solvents of increasing order of polarity. The fractions were collected in 25ml each and allowed to evaporate to get the residue. Each fraction was tested for the presence of various constituents by Thin Layer Chromatography (TLC).

#### 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°C for 1 hour and then used for TLC.
- The extracts were prepared with respective solvents like methanol, ethanol and distilled water and made up to 10 ml indifferent test tubes.
- Then with the help of capillary tube extracts was spotted on TLC plates, which were developed in TLC chamber, previously saturated with different solvent systems.

- By trial & error method, Aq. methanolic extracts showed isolation and dissolution with following solvent system.
  - Ethyl acetate : Toluene : Methanol : Glacial acetic acid :  
(7.5 : 2 : 0.5 : 0.2 )
- The different spots developed in each solvent system, the R<sub>f</sub> 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 compound

##### By TLC

Elution of compound from crude Aqueous Methanol extract

- **Colour** : Yellow sticky amorphous waxy samisolid
- **Solubility** : Water, Alcohol, Acetone
- **Melting Point** : 210<sup>0</sup> C
- **R<sub>f</sub> Value** : 0.59 (Ethyl acetate:Toluene:Methanol:Glacial acetic acid)

#### Spectral studies of isolated compound

##### By 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<sup>-1</sup>

##### 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 mass and mass of fragments, all the peaks obtained by GCMS spectroscopy are shown in figure.

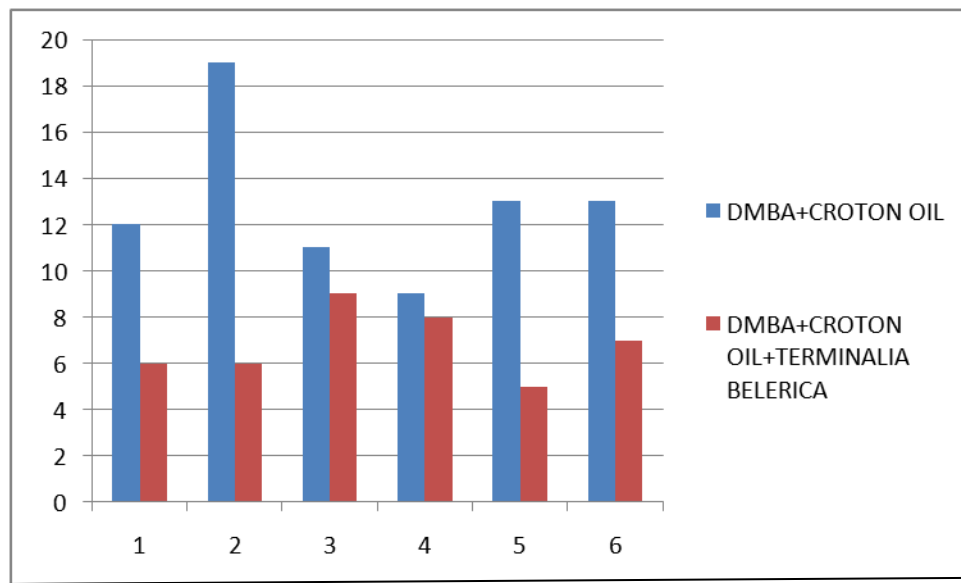
## Results and Discussion

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<sub>f</sub> 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<sub>14</sub>H<sub>2</sub>O<sub>8</sub> and molecular weight is 298.19 g/mol.

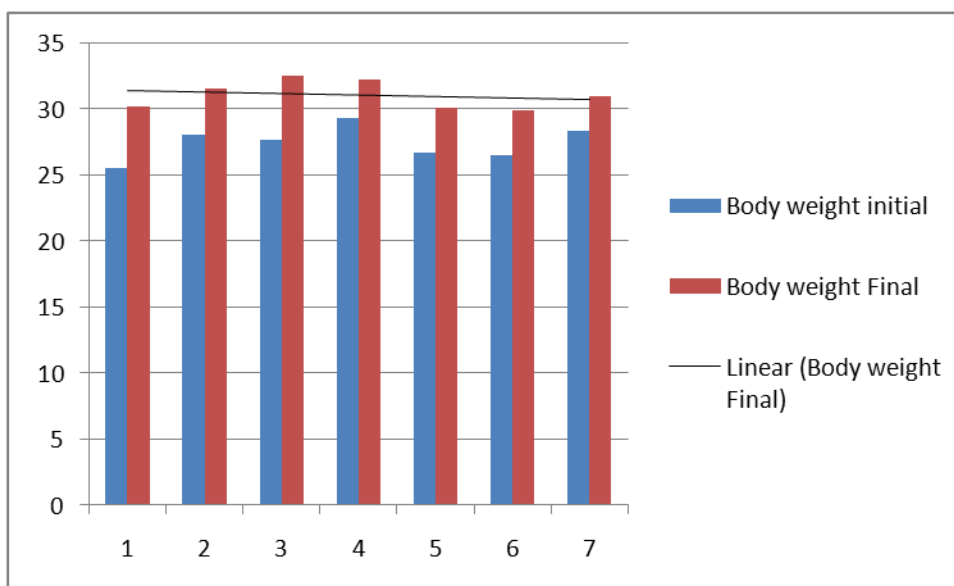
**Table No. 1 Effect of *Terminalia belerica* Roxb. On DMBA-induced papillomas in Swiss albino mice**

Groups	Body weight		No. of Papilloma (Avg.)	Tumour Yield
	Initial	Final		
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+ <i>Terminalia belerica</i> Roxb. + Croton oil	26.50	29.85	6	1.1
<i>Terminalia belerica</i> Roxb. Alone	28.30	30.95	---No---	--

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



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



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

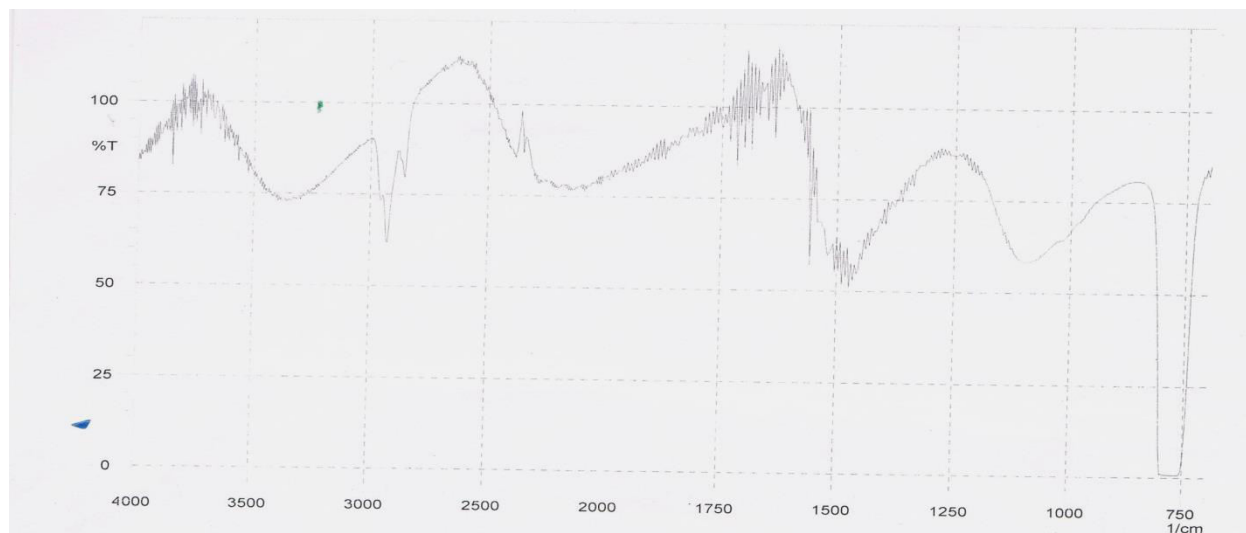
No. of fractions	Eluent	Ratio	Colour of the fraction	Rf values
F1 – F7	Petroleum ether	100	No	-
F8 – F14	Pet. Ether : Chloroform	90:10	No	-
F15 – F18	Pet. Ether : Chloroform	60:40	Greenish	0.89
F18 – F20	Pet. Ether : Chloroform	50:50	No	-
F21 – F23	Chloroform	100	No	-
F24 - F27	Chloroform : Ethyl acetate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

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 <sub>f</sub> Values
Acetone: Methanol extract of <i>Terminalia belerica</i>	Chloroform : Acetic Acid : Water  (5 : 4.5 : 0.5)	4	Yellowish Pink	0.75
D. Water Methanol extract of <i>Terminalia belerica</i>	Ethyl acetate:Toluene: Methanol: Glacial Acetic Acid  (7.5 : 2 : 0.5 : 0.2)	1	Pinkish  Light Yellow	0.59

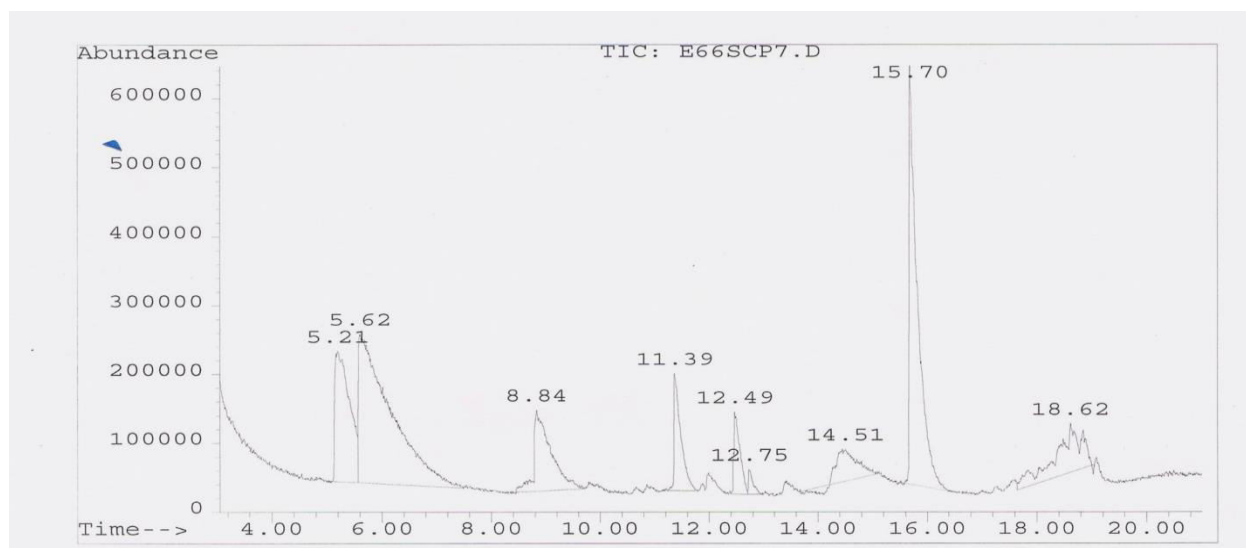
**Figure No. 3 IR Spectra of *Terminalia belerica* Roxb.**



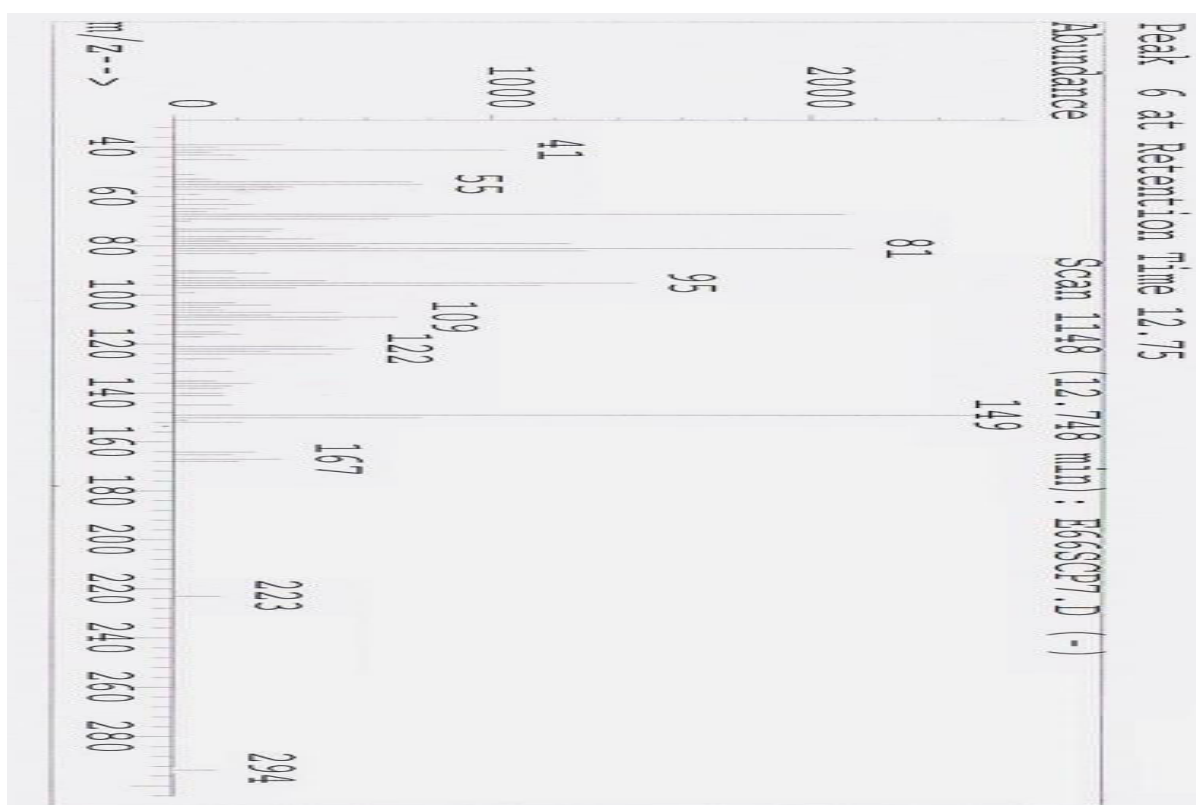
**Table No. 4 IR – Data of Isolated Compound**

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

**Figure No. 4 Gas Chromatography spectra of isolated compound**

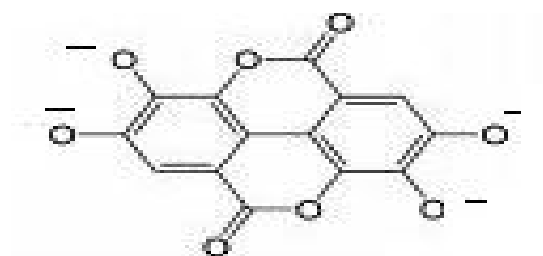


**Figure No. 5 Mass Spectrum of isolated compound**





### Structure of Isolated Compound



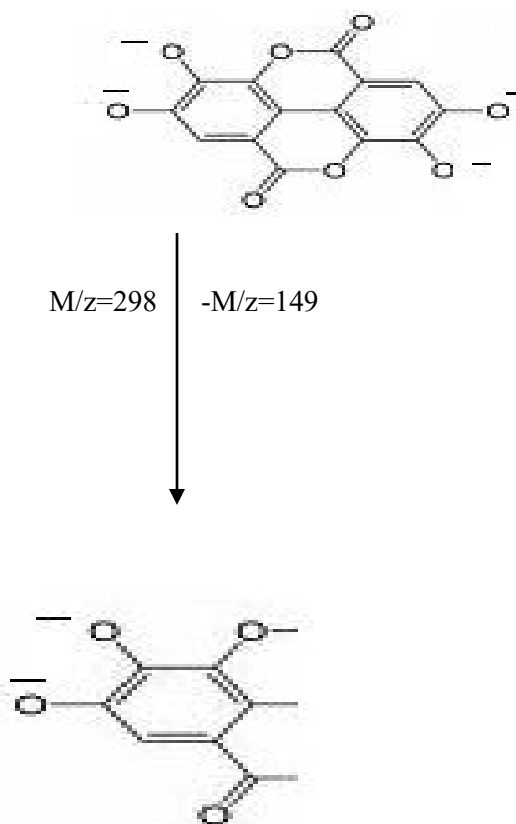
### 2, 3, 7, 8-Tetraoxy-chromeno[5,4,3-cde]chromene-5,10-dione

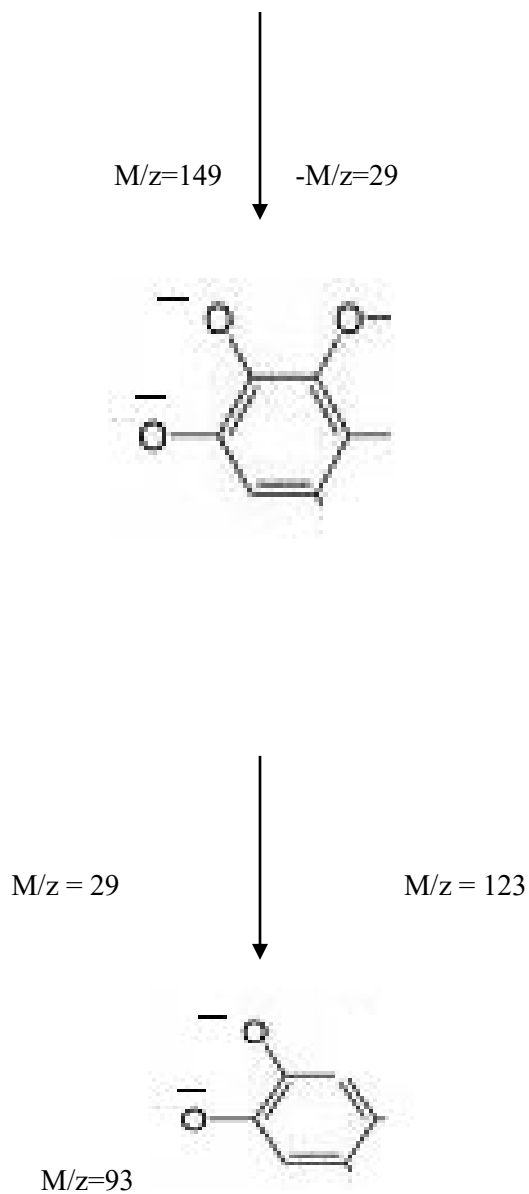
**Molecular Formula** :  $C_{14}H_2O_8$

**Molecular weight** : 298.19 g/mol

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

### Fragmentation of mass spectra of isolated compound:





### Summary and Conclusion

Based on the traditional uses and literature review of earlier studies the plant was selected. The phytochemical and pharmacological studies on *Terminalia belerica* Roxb. were done. Acute oral toxicity studies was done by following the OECD guidelines no. 402 (OECD Guidelines, 1987) showed that both acetone:methanolic and Aq. Methanolic extracts of *Terminalia belerica* Roxb. gives no effect at 2000mg/kg. Hence, 200mg/kg was taken as the effective dose. DMBA induced the papilloma cancer. DMBA dissolved 100 $\mu$ l in acetone 2 times/weeks up to 16 weeks single application was given. The DMBA was used as the cancer inducer and Croton oil 1% as cancer promoter applied on skin 2 times a week's up to 16 weeks. The dose of extract was 100  $\mu$ l. The Aqueous Methanol extracts was selected, because it has the polarity in between the Methanol and aqueous. Hence the isolation of active constituents was done

by column chromatography using various solvents according to increasing order of polarity. TLC was performed for all the fractions. In this ethyl acetate 100% gives one spot in Ethyl acetate : Toluene : Methanol : Glacial acetic acid (7.5 : 2 : 0.5 : 0.2 ) ratio. It gives  $R_f$  value at 0.59. IR, MASS spectrum of the isolated compound has been taken. From the interpretation of the data, the structure of the compound has been found. Compound 2,3, 7, 8-Tetraoxy-chromeno[5,4,3-cde]chromene-5,10-dione was isolated and its structure was characterized by interpreting spectral data. Further phytochemical and pharmacological studies on the extract *Terminalia belerica* Roxb. may lead to significant outcome.

### Acknowledgements

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