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EVALUATION OF SEEDS OF AN INDIAN SACRED PLANT *AEGLE MARMELOS*, FOR THEIR ANTIOXIDANT AND CYTOTOXIC POTENTIAL

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Keywords:

Aegle marmelos seeds, Antioxidant activity, Cytotoxic activity, Flavonoids

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ABSTRACT: Introduction: The seeds of *Aegle marmelos* (bael) are less explored for their therapeutic activity; cytotoxic activity of bael seeds was yet to be studied. The present study aims at finding the cytotoxic activity of the successive extracts and the flavonoid fraction from the methanolic extract of the seeds. **Methods:** The extracts were prepared using solvents (petroleum ether, chloroform, ethyl acetate, methanol, and water) in increasing order of their polarity. Anti-oxidant activity of the extracts was evaluated using DPPH radical scavenging assay, nitric oxide scavenging assay, and hydrogen peroxide scavenging assay. Flavonoid fractions were obtained by thin-layer chromatography of the flavonoid-rich fraction prepared from the total methanolic extract. Human colon cancer cell line (HT 29), human breast cancer cell line (MCF-7), human lung cancer cell line (A-549) were used to evaluate the cytotoxicity of the extracts and the flavonoid fractions. **Results:** Various phytochemicals like alkaloids, flavonoids, tannins and phenols, steroids, and saponins were detected in phytochemical investigation of the extract. Ethyl acetate fraction showed comparable DPPH radical scavenging activity and hydrogen peroxide scavenging activity with ascorbic acid (standard). Methanol extract showed good nitric oxide scavenging activity of all the extracts. Flavonoid fraction F-01 showed 50% cell growth inhibition at concentration <10 µg/ml on the human breast cancer cell line (MCF-7), which indicates its potent activity. **Conclusion:** F-01, one of the flavonoid fractions, showed comparable activity with the standard (Adriamycin) on MCF-7 cell line. Anti-oxidant study was conducted for the extracts, and the extracts showed good activity comparable to the standards used.

INTRODUCTION: Traditional medicine is well known and used for ages due to less side effects after its administration. One such medicinal plant used in India since ancient times is *Aegle marmelos*.

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Aegle marmelos, commonly known as Bael belong to the Rutaceae family. The plant is precious to the Hindu god Lord Shiva, and its leaves are offered as offering to Lord Shiva. It is the most ancient documented plant in India, according to Charak (1500 BC) [1].

Charak Samhita, prominent ayurvedic literature, describes bael as an essential ingredient in the ayurvedic system of medicine [1]. Although native to Northern India, it is widely distributed throughout the Indian subcontinent, Sri Lanka, Pakistan, Bangladesh, Burma, Thailand and most south-

eastern Asian countries [2]. It is a mid-sized tree that grows up to 15m tall. Every part of the tree, *i.e.*, leaf stem bark, root, fruit and seeds, have medicinal values.

Bael is used as a carminative, laxative, astringent and stomachic and also treats conditions like fever, diarrhea, vomiting, dysentery, diabetes, asthma, inflammation, *etc*.

Unripe bael fruits are bitter, astringent, and haveanti-laxative properties, improve digestion and treat diarrhea. Ripe fruits are sweet, aromatic, and astringent with laxative properties [3, 4]. *Aegle marmelos* are known by various names in different Indian languages, some listed in **Table 2**.

There are marketed products of bael, like jam, chyawanprash, squash, bael powder, capsules, syrup and tablets [5]. **Table 1** provides the taxonomic classification of *Aegle marmelos*.

TABLE 1: TAXONOMIC CLASS OF *AEGLE MARMELOS* [6]

Taxonomic Classification	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	*Aegle* Corr. Serr.
Species	*Aegle marmelos* (L.) Corr. Serr.

TABLE 2: NAMES OF *AEGLE MARMELOS* IN VARIOUS INDIAN LANGUAGES [7]

Language	Vernacular name
Hindi, Marathi	Bel, Belgiri and Bili
Gujarati	Bilavaphal
Urdu	Belk ham
Oriya	Belo
Tamil	Vilva marum or Vilvama
Sanskrit	Shivaphala
Malayalam	Marredy

TABLE 3: LITERATURE REVIEW OF VARIOUS PARTS OF *A. MARMELOS*

Extract/Active Compound	Activity	Animal model /Cell lines	Year	References
Leaf				
Leaf juice	Hypoglycemic activity	Patients	2021	8
Successive leaf extracts	Anticancer activity	MDA-MB-231,Vero cells, HEp-2	2019	9
Methanolic and aqueous leaf extracts	Antioxidant activity	*In-vitro* antioxidant tests	2019	10
Methanolic leaf and fruit extract	Antihepatotoxic activity		2018	11
Sequential extraction Chloroform, ethyl acetate, acetone, methanol; Aqueous extract	Antibacterial, Antifungal, Antioxidant and Cytotoxic Properties	Antibacterial, antifungal activity-Various strains of bacteria and fungi Cytotoxicity-MTT assay using T47D cell line	2017	12
Ethanolic extract of leaf and fruit pulp	Anti-inflammatory and analgesic activity	Wistar albino rats	2017	13
Ethanolic leaf extract	Against cognitive and neurological disorder	Albino Wistar rats	2019	14
Fruit				
Ethanolic and aqueous extract	Anti-microbial		2018	15
Aqueous Fruit extract	Antioxidant, anticancer, antibacterial activity	In vitro anti-oxidant tests, Bacterial strains, MCF-7 cell line	2018	16
Seed				
Aqueous seed extract	Hypoglycemic and antihyperglycemic activity	Male albino wistar rats	2006	17
1-Methyl-2-(3'-methyl-but2'-enyloxy)-anthraquinone from methanolic extract of seed	Antifungal activity	*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*	2010	18
Methanolic seed extract	Anti-inflammatory activity	Male Wistar rats	2011	19

Literature survey **Table 3** reports various activities of different parts of *Aegle marmelos*. Leaf, fruit, bark and seeds are evaluated for their activities using various solvents. Therapeutic activities like antidiabetic, antifungal, antimicrobial, and anti-inflammatory activity of seeds are reported in the literature. Preclinical studies have shown that bael leaf extracts effectively inhibit the growth of

leukemic K562, T-lymphoid, B-lymphoid, erythroleukemic HEL, melanoma Colo 38 and breast cancer cell lines MCF-7 and MDA-MB-231. Seeds are evaluated for their anti-inflammatory, hypoglycemic and anti-hyperglycemic, antidiabetic and antifungal activities. There are no reports of studies on seeds for cytotoxic potential to date as explored on databases like pubmed, medscape, google scholar, and a gray area. Present research work aims on finding the cytotoxicity of the extracts and the flavonoid fractions.

EXPERIMENTAL:

Collection and Authentication of Plant Material: *Aegle marmelos* (Bael) seeds were procured from Greenfield Agro forestry products, Madhya Pradesh, India. Procured seeds were authenticated from Blatter Herbarium, St. Xavier's College, Mumbai, India. It was found that the specimen matched with Blatter Herbarium specimen number Bole-21 P. V. Bole and hence authenticated and certified that the specimen was of *Aegle marmelos*.

Preparation of Extracts: The seeds were shade dried and ground into a coarse powder. 80 gm of seeds was defatted using petroleum ether. The powder was then successively extracted using the Soxhlet apparatus using solvents to increase polarity: petroleum ether, chloroform, ethyl acetate, methanol, and distilled water.

Qualitative Phytochemical Investigation: The plant extracts were examined for the presence of alkaloids, flavonoids, tannins and phenols, glycosides, saponins, and steroids as per standard protocols [20].

Antioxidant Studies:

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity of the extract was determined by the method reported by Awah *et al.* [21] 2ml of different concentrations (20, 40, 60, 80,100 ppm) of the extracts were prepared and added to 1ml of 0.3 mM DPPH prepared in ethanol. The resulted solution was agitated and left to stand for 25 min at room temperature. 2ml of test solutions and 1 ml of ethanol were mixed to prepare blank solutions and 1 ml of 0.3mM DPPH solution and 2 ml of ethanol were mixed to prepare negative control. L-Ascorbic acid was used as the positive control.

The absorbance of the solutions was measured at 518 nm against each blank using a UV Vis Diode Array spectrophotometer. The assay was done in triplicate. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = A_{control} - A_{test} / A_{control} \times 100$$

Nitric Oxide Radical Scavenging Assay: The activity of the extracts was determined by the method reported by Boora *et al.* with some modifications [22]. 1000 ppm stock solutions of the extracts were preparedin ethanol. The extracts were diluted to prepare concentrations ranging from 20-100 ppm (20, 40, 60, 80, 100 ppm). Gallic acid was used as standard. To prepare the Griess reagent, equal volumes of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) and 1% sulphanilamide in 2.5% phosphoric acid were mixed and used immediately.

10mM sodium nitroprusside solution was prepared in phosphate-buffered saline and 0.5 ml of this solution was mixed with 1ml of different concentrations of the extracts and incubated for 180 mins at 25°C. The incubated solutions were mixed with equal proportions of freshly prepared Griess reagent. Control samples were prepared similarly but without the extracts. The absorbance of the samples was measured at 546nm using a UV Vis Diode Array spectrophotometer. The assay was done in triplicate. Percent inhibition of the extracts and the standard was calculated using following formula:

$$\text{Nitric oxide Scavenged (\%)} = A_{control} - A_{test} / A_{control} \times 100$$

Hydrogen Peroxide Scavenging Assay: The scavenging activity of the extracts toward hydrogen peroxide radicals was determined by the method by Ngonda *et al.* [23] 40mM solutions of hydrogen peroxide was prepared in phosphate buffer (ph 7.4). Extracts of different concentrations (50, 100, 150, 200, 250, 300 ppm) were prepared. 2ml of hydrogen peroxide was added to 1 ml of extracts and incubated for 30min. Absorbance was recorded at 560 nm. Ascorbic acid was used as the standard. The assay was done in triplicate. Percent scavenging activity was calculated using the following formula:

$$\text{Percent scavenged } (H_2O_2) = 1 - \text{Absorbance (standard)} / \text{Absorbance (control)} \times 100$$

Total Flavonoid Content of Methanolic Extract: Total flavonoid content of the methanolic extract was determined by the method by Chang *et al.* with slight modifications [24]. Quercetin was used to obtain a calibration curve. A 1000 ppm stock solution of quercetin was prepared in 80% ethanol. Dilutions were made to prepare 6.25, 12.5, 25, 50, 80, 100 ppm solutions from the stock solution. Each 0.5ml of diluted sample was mixed individually with 1.5 ml of 95% methanol, 0.l ml of AlCl3 (aluminum chloride), 0.1 ml of 1M CH_3CO_2K (potassium acetate), and 2.8 ml of distilled water. The samples were incubated for 30mins at room temperature. The absorbance of the sample solutions was measured at 415 nm using UV spectrophotometer. For the blank solution, aluminium chloride was replaced with the same amount of distilled water. On similar lines, 0.5 ml of methanolic extract was made to react with aluminium chloride to estimate total flavonoid content.

$$C = (c \times V) / m$$

C = Flavonoid content (QE/g) c = Concentration of quercetin from calibration curve (mg/mL) V= Volume of extract (mL) m = weight of plant extract (g)

Preparation of Flavonoid Rich Fraction (FRF): Total methanolic extract was prepared. The extract was suspended in distilled water. The resulting aqueous solution was partitioned with petroleum ether using a separating funnel.

The petroleum ether fraction was discarded the aqueous fraction was further separated with ethyl acetate. The aqueous fraction was discarded, and the ethyl acetate fraction was concentrated to obtain the flavonoid-rich fraction. The percent extractive yield of the flavonoid-rich fraction was determined [25].

Thin Layer Chromatography of FRF: Thin layer chromatography of FRF was performed using the mobile phase Ethyl acetate: Formic acid: Glacial acetic acid: Water: 10: 0.5: 0.5: 1.5. The obtained bands (silica) were scraped off from the TLC plate. They were suspended in ethyl acetate.

The suspension was filtered using a Whatman filter paper. The filtered fractions were concentrated in a water bath.

LC-MS Analysis of Isolated Flavonoid Fractions: The isolated flavonoid fractions (Band 1, Band 2, Band 3, Band 4) 10mg(approx.) were subjected to LC-MS analysis at IIT SAIF, Mumbai-400076 (LC-MS; Agilent technologies; ES-API scan).

***In-vitro* Cytotoxic Studies:** The plant extracts were evaporated to dryness under a rotary evaporator. The concentrated extracts, the flavonoid-rich extract, and the isolated flavonoid fractions were subjected to *in-vitro* cytotoxicity studies on MCF-7, HT-29 and A-549 cell lines at ATREC (The Advanced Centre for Treatment, Research and Education in Cancer).

RESULTS:

Preparation of Extracts: The extracts were prepared successively. The excess solvents were recovered using the Soxhlet apparatus. The extract was concentrated using a water bath. **Table 4** shows the percent yield of the extracts obtained.

TABLE 4: PERCENT YIELD OF THE EXTRACTS OBTAINED USING VARIOUS SOLVENTS

Solvents	% Yield
Petroleum ether	35.66%
Chloroform	1.25%
Ethyl acetate	0.225%
Methanol	6.36%
Water	0.58%
Total Methanolic extract	2.9%

Qualitative Phytochemical Investigation: Phytochemical investigation of the extracts was performed qualitatively using standard procedures in which chloroform extract shows the presence of the alkaloids, flavonoids, tannins and phenols, saponins. Ethyl acetate extract showed the presence of flavonoids, tannins, phenols and saponins.

TABLE 5: PHYTOCHEMICAL INVESTIGATION OF THE SUCCESSIVE EXTRACTS

Solvents	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol	Water	Total Methanolic Extract
Alkaloids	-	+	-	-	-	-
Flavonoids	-	+	+	+	-	+
Tannins and phenols	-	+	+	+	-	+
Glycosides	-	-	-	-	-	-

Saponins	-	+	+	+	+	+
Steroids	+	-	-	+	-	

Present: + Absent: -

Methanol extract showed positive tests for flavonoids, tannins and phenols, saponins and steroids. Total methanolic extract showed the presence of flavonoids, tannins and phenols, and saponins. Petroleum showed the presence of steroids. **Table 5** summarizes the results of phytochemical tests.

Antioxidant Studies:

DPPH Assay: DPPH assay was performed, ethyl acetate extract showed better DPPH radical scavenging activity of all the extracts (IC_{50}= 27.2 µg/ml) comparable with that of the standard ascorbic acid (IC_{50}= 22.5 µg/ml). **Table 6** gives the IC_{50} values of the extracts and the standard ascorbic acid. **Fig. 1** shows a graphical representation of the DPPH radical scavenging activity of the extracts and the standard.

TABLE 6: DPPH RADICAL SCAVENGING ACTIVITY OF STANDARD AND EXTRACTS

Extract / Control	IC_{50} (µg/ml)
Ascorbic acid	22.5
Petroleum ether	49.6
Chloroform	34.2
Ethyl acetate	27.2
Methanol	30.1
Distilled water	45.4

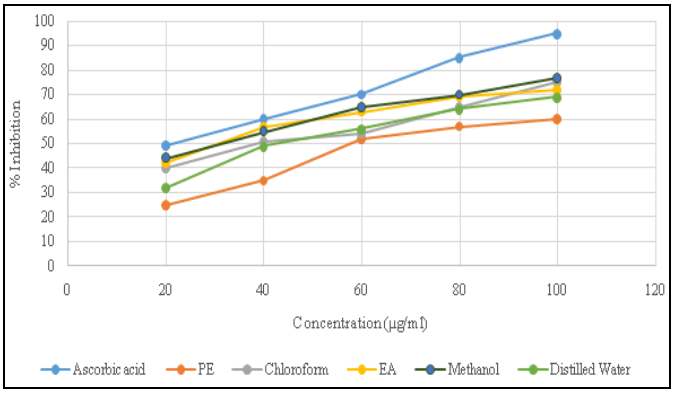

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY

Nitic Oxide Scavenging Assay: Methanolic extract scavenged more nitric oxide (IC_{50} =22.6 µg/ml) as compared to other extracts and showed comparable activity with the standard gallic acid (IC_{50} = 20.8 µg/ml). IC_{50} values of extracts and the standard for nitric oxide scavenging activity is given in **Table 7,** and a graphical representation of the same is shown in **Fig. 2.**

TABLE 7: NITRIC OXIDE SCAVENGING ACTIVITY OF STANDARD AND EXTRACTS

Extract	IC_{50} (µg/ml)
Gallic acid	20.8
Petroleum ether	45.3
Chloroform	30.4
Ethyl acetate	24.2
Methanol	22.6
Distilled water	40.8

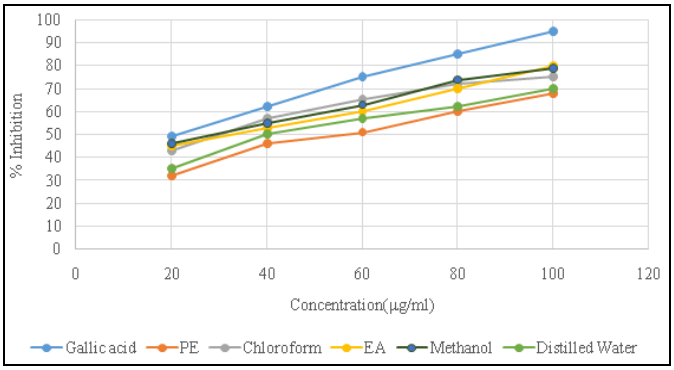

FIG. 2: NITRIC OXIDE SCAVENGING ACTIVITY

Hydrogen Peroxide Scavenging Assay: Hydrogen peroxide scavenging activity was shown best by ethyl acetate (IC_{50}= 180.5 µg/ml) compared to other extracts. The IC_{50} value of the standard ascorbic acid was found to be 150.3µg/ml. **Table 8** gives the hydrogen peroxide scavenging assay result, and **Fig. 3** gives its graphical representation.

TABLE 8: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF STANDARD AND EXTRACTS

Extract	IC_{50} (µg/ml)
Ascorbic acid	150.3
Petroleum ether	250.5
Chloroform	208.2
Ethyl acetate	180.5
Methanol	190.3
Distilled water	200.2

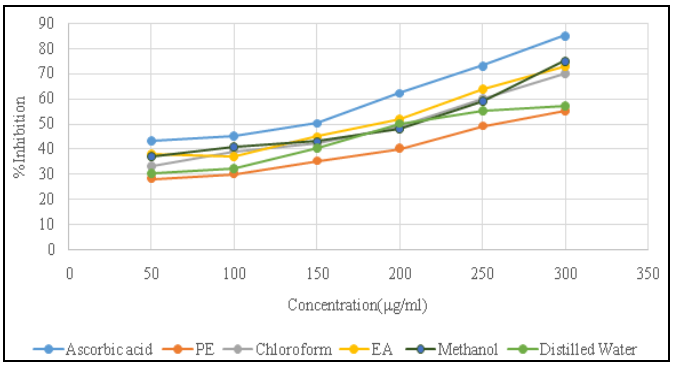

FIG. 3: HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Total Flavonoid Content: Flavonoid content of methanolic extract by aluminium chloride method was found to be 12.933 mg of QE/g. **Fig. 4** shows the calibration curve of quercetin. The total flavonoid content was calculated to be 12.933 mg of QE/g.

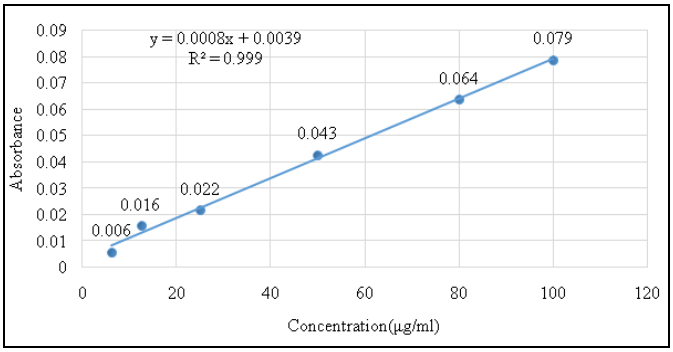

FIG. 4: CALIBRATION CURVE OF QUERCETIN

Preparation of Flavonoid Rich Fraction: The percent yield of the flavonoid-rich fraction was 14%w/w.

Thin Layer Chromatography of FRF: Four distinct fluorescent bands were obtained on the TLC plate under long UV exposure. Rf of these bands were 0.129 (Band 1), 0.17 (Band 2), 0.612 (Band 3), and 0.79 (Band 4). **Fig. 5** shows the thin layer chromatography plate under long UV exposure showing 4 distinct flavonoid bands.

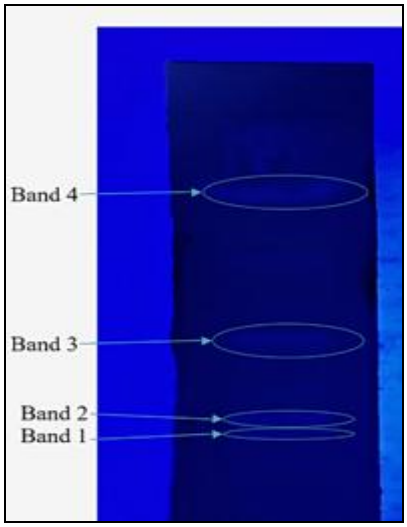

FIG. 5: THIN LAYER CHROMATOGRAPHY OF FLAVONOID-RICH FRACTION

LC-MS Analysis of Isolated Flavonoid Fractions:

The LC-MS analysis of the fractions showed the presence of the following compounds:

Fraction 01: Fig. 6-10.

- Annofoline
- Scopoletin
- Agyrolobine
- Luvangetin
- Fencamfamine

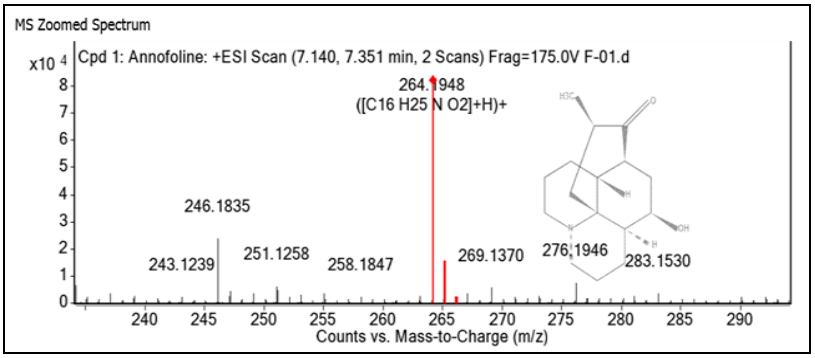

FIG. 6: MASS SPECTRUM OF ANNOFOLINE

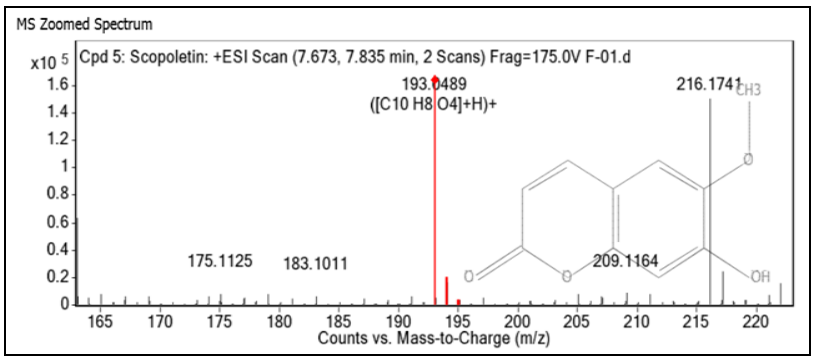

FIG. 7: MASS SPECTRUM OF SCOPOLETIN

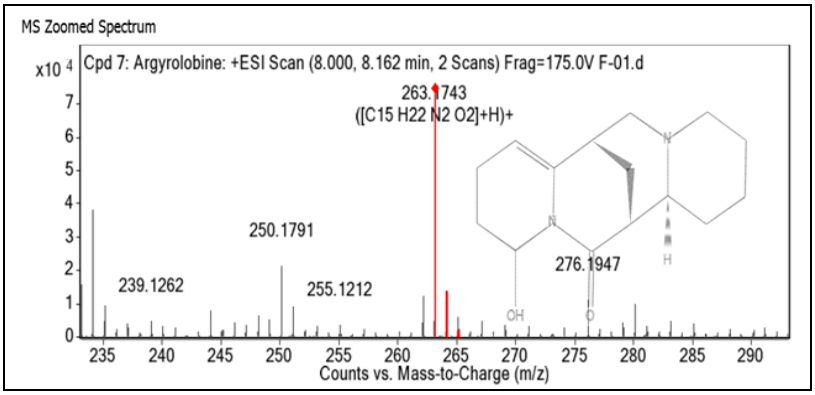

FIG. 8: MASSSPECTRUM OF AGYROLOBINE

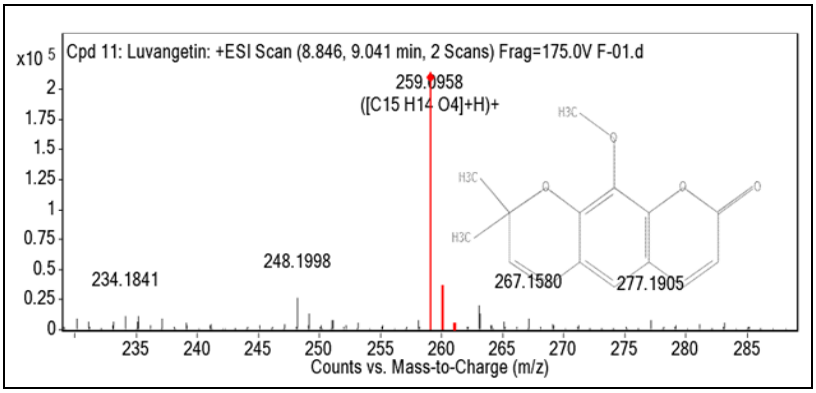

FIG. 9: MASS SPECTRUM OF LUVANGETIN

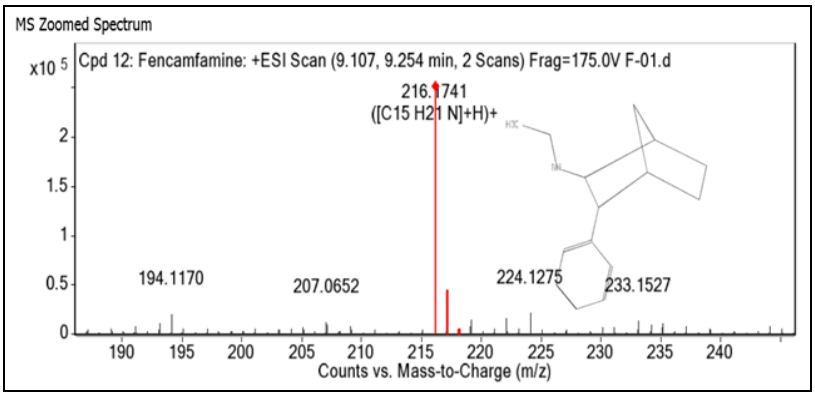

FIG. 10: MASS SPECTRUM OF FENCAMFAMINE

Fraction 02: Fig. 11-15.

- L-Galactose
- 11-amino-undecanoic acid
- 6-Hydroxyangelicin
- Formononetin
- Withaphysacarpin

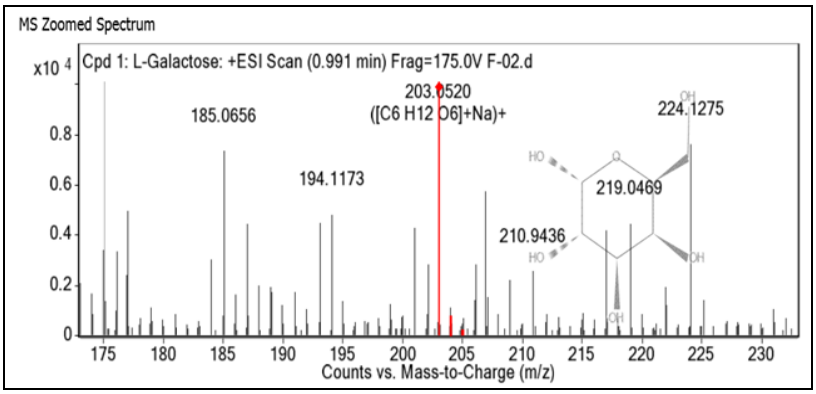

FIG. 11: MASS SPECTRUM OF L-GALACTOSE

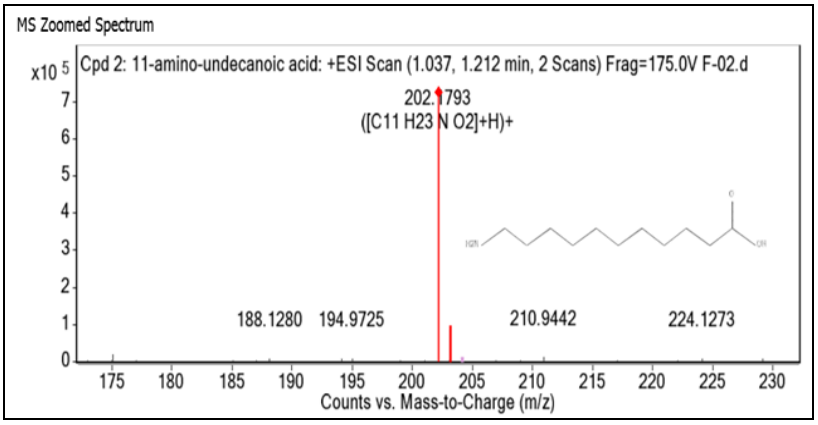

FIG. 12: MASSSPECTRUM OF 11-AMINO-UNDECANOIC ACID

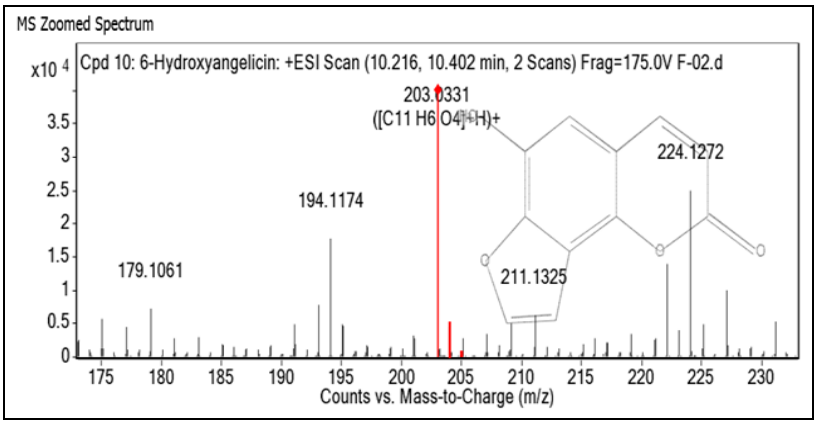

FIG. 13: MASS SPECTRUM OF 6-HYDROXYANGELICIN

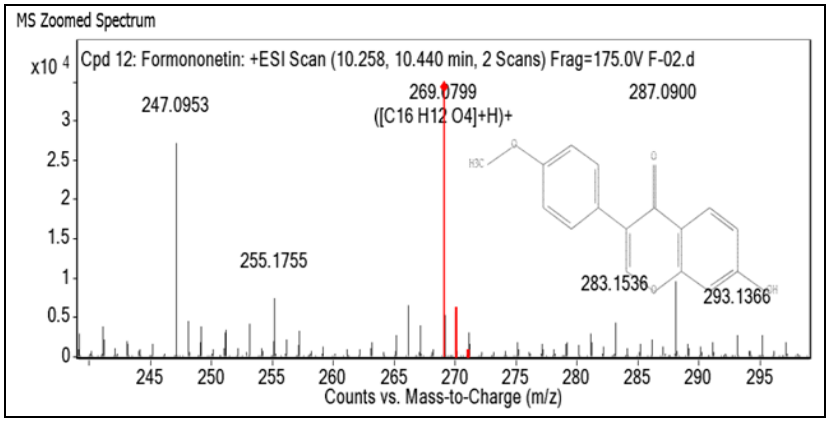

FIG. 14: MASS SPECTRUM OF FORMONONETIN

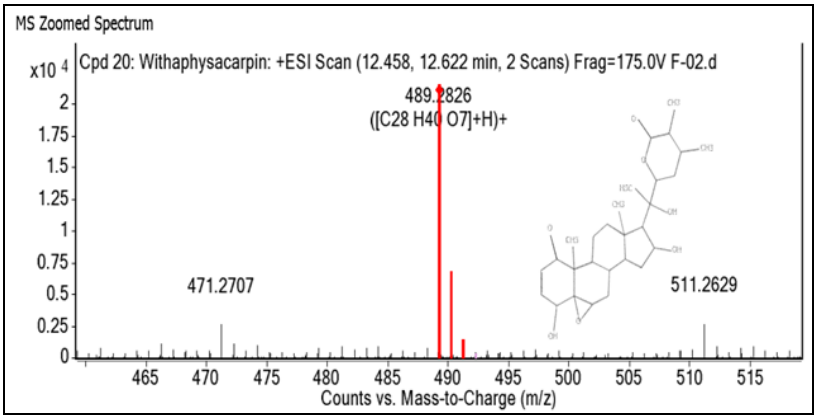

FIG. 15: MASSSPECTRUM OF WITHAPHYSACARPIN

Fraction 03: Figure 16-20.

- Lycoflexine
- Carnegine
- Trihydroxycoprostanoic acid
- Isobergaptol
- D8'-Merulinic acid A

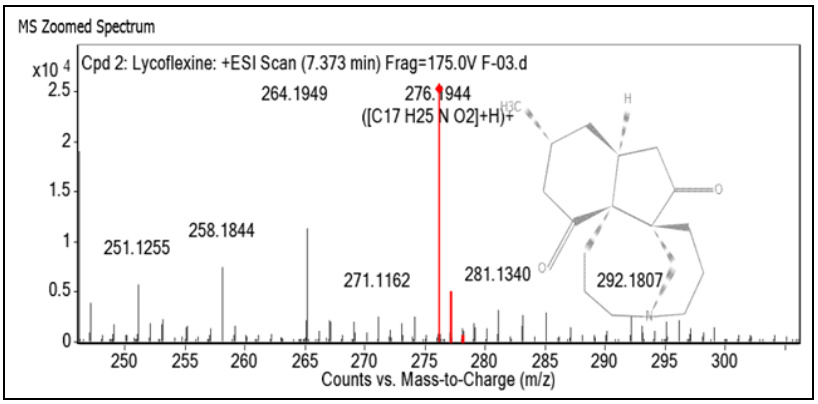

FIG. 16: MASS SPECTRUM OF LYCOFLEXINE

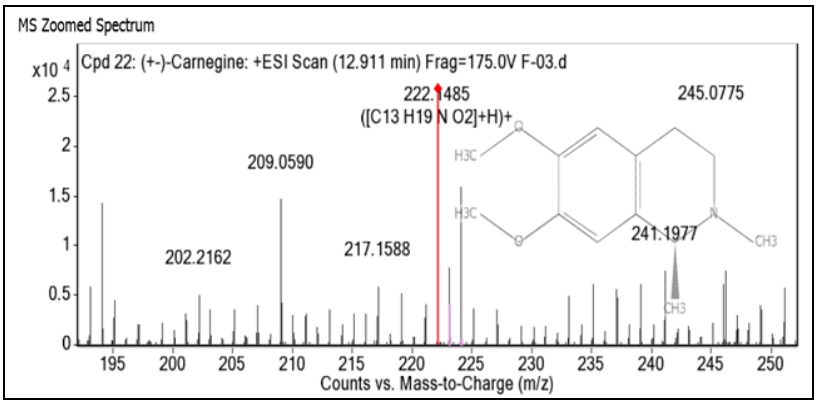

FIG. 17: MASS SPECTRUM OF CARNEGINE

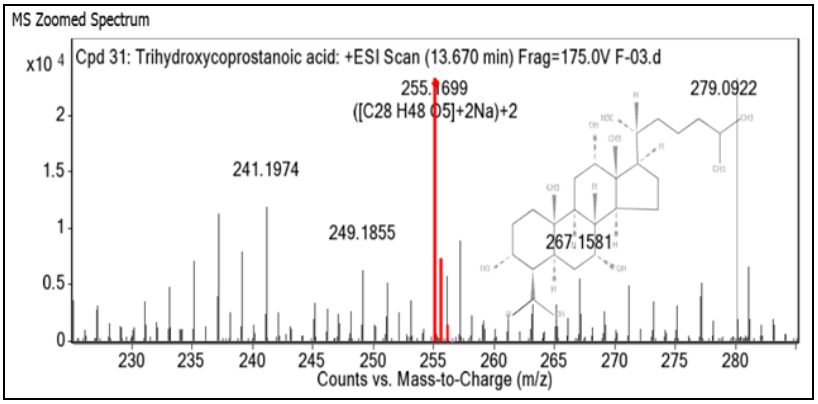

FIG. 18: MASSSPECTRUM OF TRIHYDROXYCOPROSTANOIC ACID

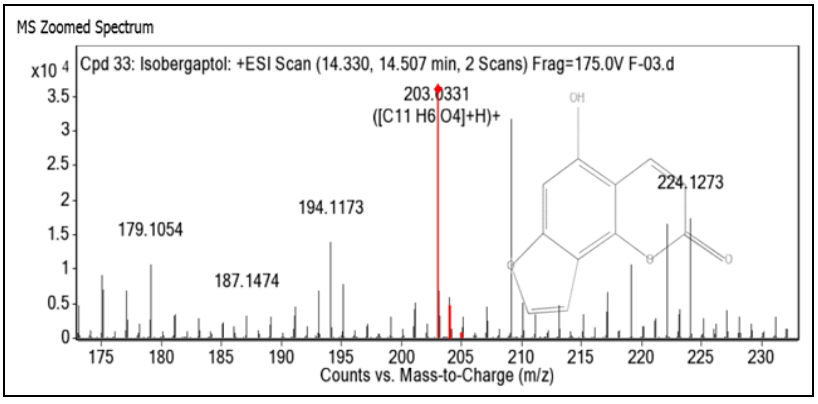

FIG. 19: MASS SPECTRUM OF ISOBERGAPTOL

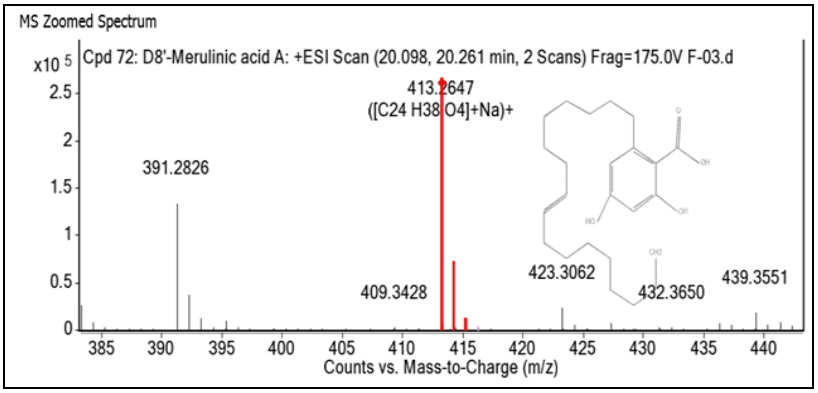

FIG. 20: MASSSPECTRUM OF D8'-MERULINIC ACID A

Fraction 04: Fig. 21-25.

- ❖ 1-(5-Methyl-3pyridinyl)-1-decanone
- ❖ (±)-2-Methyl-3-(4methylphenyl)propanal
- ❖ Alnustone
- ❖ Chloropanaxydiol
- ❖ Phenanthrene

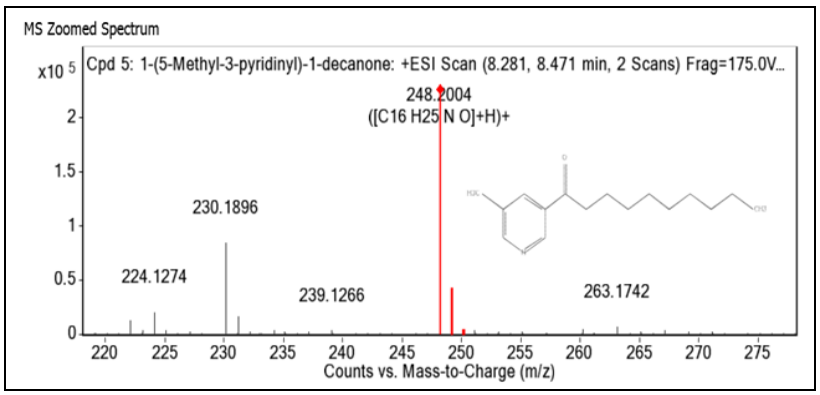

FIG. 21: MASSSPECTRUM OF 1-(5-METHYL-3PYRIDINYL)-1-DECANONE

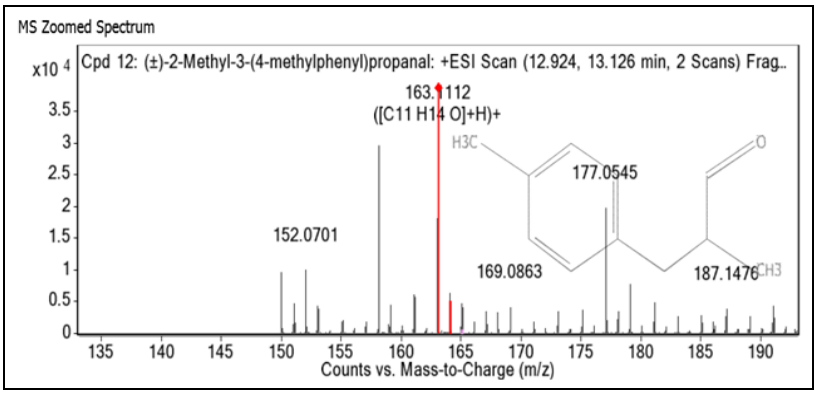

FIG. 22: MASSSPECTRUM OF (±)-2-METHYL-3-(4METHYLPHENYL) PROPANAL

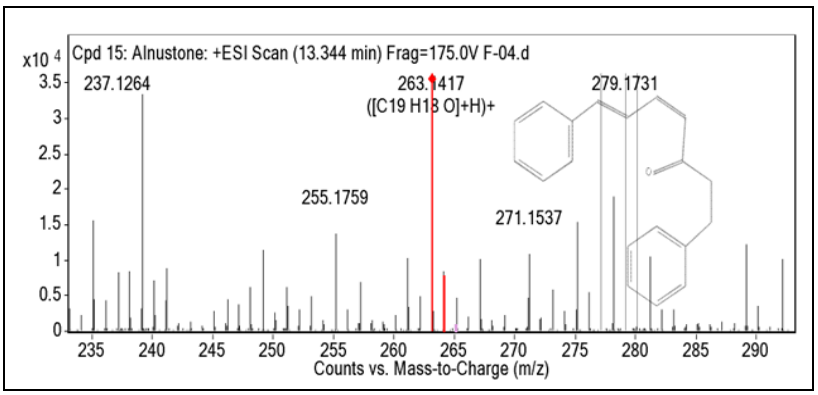

FIG. 23: MASS SPECTRUM OF ALNUSTONE

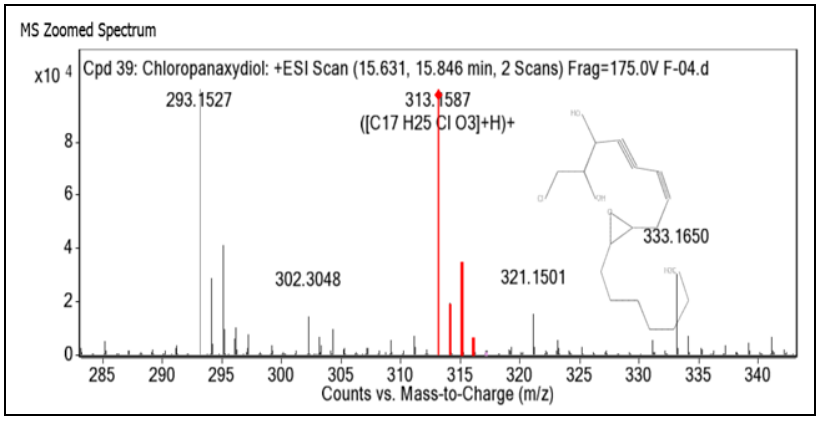

FIG. 24: MASS SPECTRUM OF CHLOROPANAXYDIOL

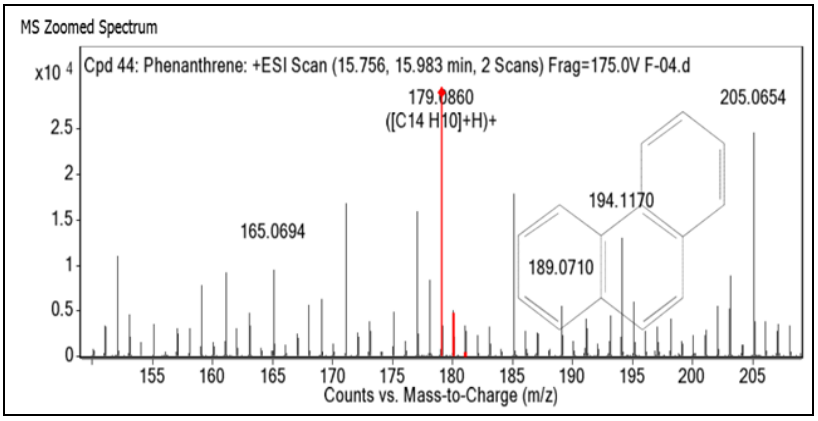

FIG. 25: MASS SPECTRUM OF PHENANTHRENE

***In-vitro* Cytotoxicity Studies:**

Human Breast Cancer Cell Lines (MCF-7):

In-vitro cytotoxicity study of extracts and flavonoid fractions on MCF-7 human breast cancer cell line revealed that flavonoid fraction F-01 showed comparable with the standard, Adriamycin. F-01 and Adriamycin showed activity at concentration <10µg/ml. All the extracts, FRF and fractions F-02, F-03, F-04 showed activity at concentration >80 µg/ml. **Fig. 26** gives the growth curve of the extracts, FRF and the flavonoid fractions on human breast cancer cell lines(MCF-7).

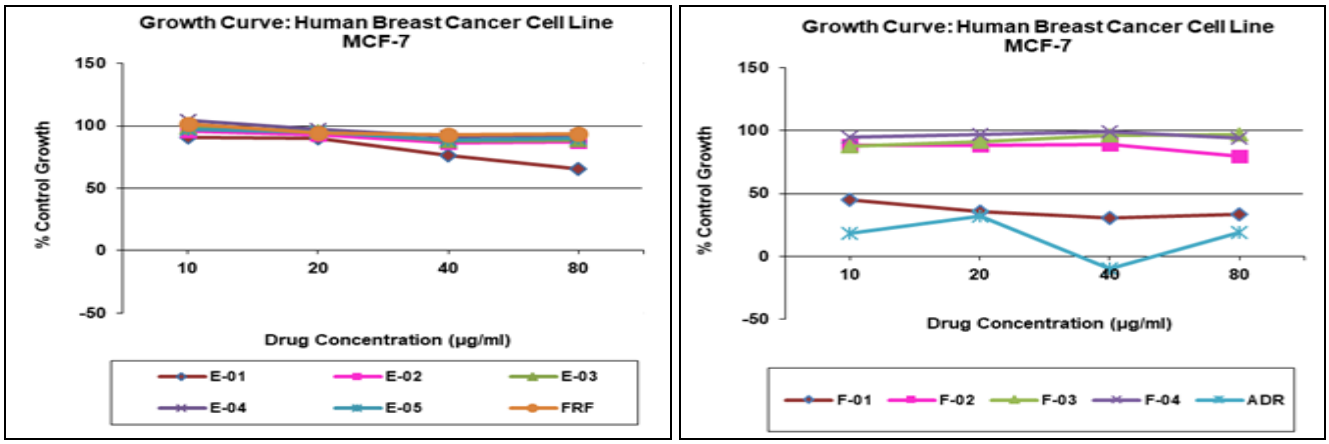

FIG. 26: GROWTH CURVE: HUMAN BREAST CANCER CELL LINE MCF-7

Human Colon Cancer Cell Line (HT-29): In the *in-vitro* cytotoxicity study on human colon cancer cell line HT-29, all the extracts, FRF and the flavonoid fractions showed activity at concentration > 80 µg/ml. Adriamycin, the standard, showed activity at concentration <10µg/ml. **Fig. 27** gives the growth curve of the extracts, FRF and the flavonoid fractions on human colon cancer cell line (HT-29).

Human Colon Cancer Cell Line (HT-29):

In the *in-vitro* cytotoxicity study on human colon cancer cell line HT-29, all the extracts, FRF, and the flavonoid fractions showed activity at concentrations> 80 µg/ml. Adriamycin, the standard, showed activity at a concentration <10µg/ml. **Fig. 27** gives the growth curve of the extracts, FRF, and the flavonoid fractions on the human colon cancer cell line (HT-29).

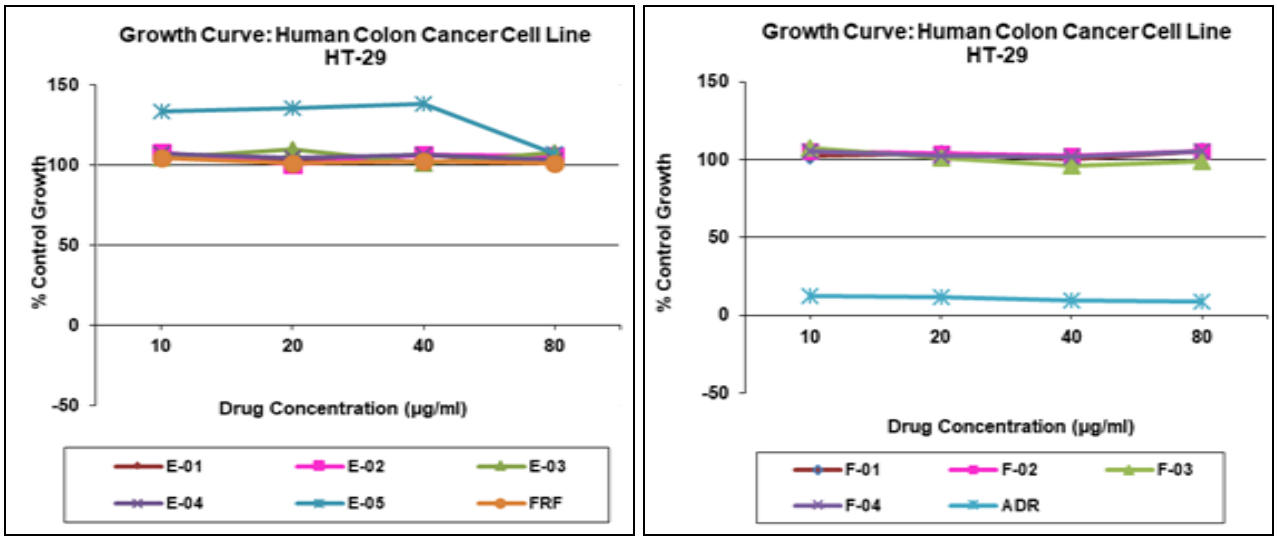

FIG. 27: GROWTH CURVE: HUMAN COLON CANCER CELL LINE HT-29

Human Lung Cancer Cell Line (A-549):

In the *in-vitro* cytotoxicity study on human lung cancer cell line A-549, the standard, Adriamycin, showed activity at <10µg/ml. All the extracts, FRF, and flavonoid fractions showed activity at concentrations>80 µg/ml. **Fig. 28** gives the growth curve of the extracts, FRF, and the flavonoid fractions on human lung cancer cell line(A-549).

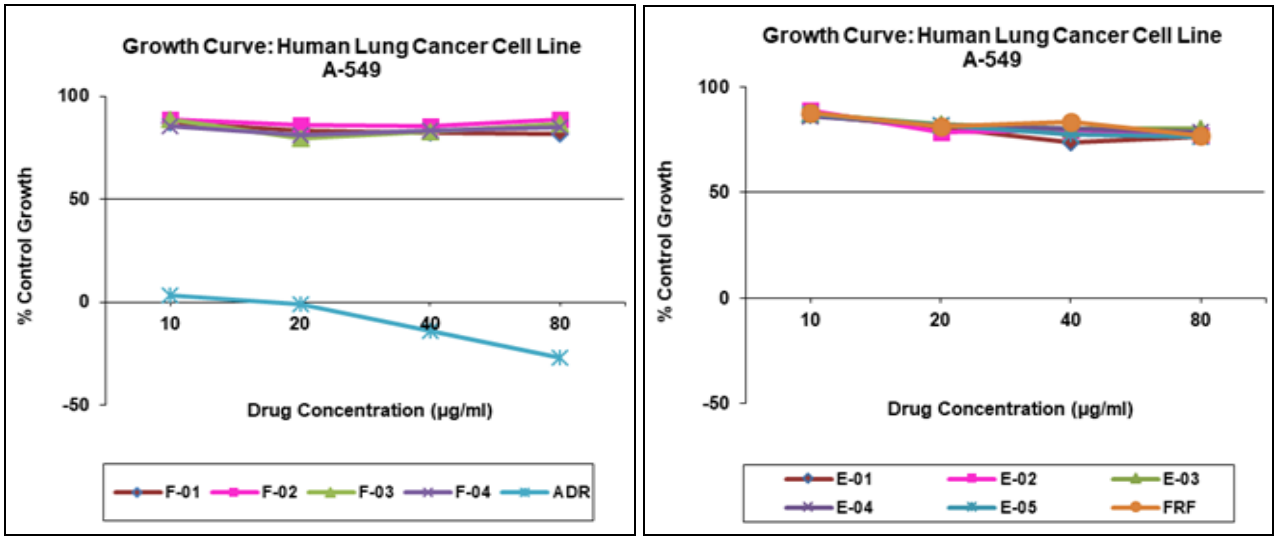

FIG. 28: GROWTH CURVE: HUMAN LUNG CANCER CELL LINE A-549

DISCUSSION: Phytochemical screening of the plant extracts were prepared using methanol and solvents in the increasing order of their polarity (petroleum ether, chloroform, ethyl acetate, methanol, and distilled water). The extracts were evaluated for their antioxidant potential. DPPH radical scavenging assay, nitric oxide scavenging assay and hydrogen peroxide scavenging assay were performed. The total methanolic extract's total flavonoid content was estimated using aluminium chloride method. The flavonoid-rich extract was prepared from the total methanolic extract. Thin-layer chromatography was performed using a solvent system (Ethyl acetate: Formic acid: Glacial acetic acid: Water:: 10: 0.5: 0.5: 1.5). Four distinct flavonoid bands were obtained using TLC when

observed under long wavelength UV radiation. LC-MS scan was performed for the flavonoid bands obtained through TLC. Cytotoxicity studies of the extracts and flavonoid fractions were performed on the cells lines: MCF-7, HT-29, and A-549.

Limitations: While *in-vitro* studies were performed in this study, animal models can be used to study antioxidant and cytotoxic potential of the extracts and the fractions. More sensitive methods of characterization like GC-MS can be used.

CONCLUSION: Extracts showed various phytochemicals like flavonoids, tannins and phenols, saponins, and alkaloids. Only petroleum ether extract showed the presence of steroids. Chloroform extract showed the presence of alkaloids, flavonoids, tannins and phenols, and saponins. Ethyl acetate extract and methanol extract showed the presence of flavonoids, tannins and phenols, and saponins. Ethyl acetate and methanol extract showed good antioxidant potential among all the extracts. LC-MS data from the existing database was acquired from the fractions obtained using TLC. F-01 fraction showed comparable activity with the standard (Adriamycin) on the MCF-7 cell line.

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Data Availability: No supplementary file submitted.

CONFLICTS OF INTEREST: Nil

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