Review Article

Anticancer Activity, Phytochemical Screening, Isolation and Structure Elucidation of Scopoletin in *Ipomoea reniformis* Choise: A Review

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ABSTRACT

The quality of herbal medicines used to treat various cancers has significantly increased because to extensive pharmaceutical research. We are now much better equipped to detect many anticancer plants thanks to the development of molecular science and the improvement of isolation and structural elucidation tools. *Ipomoea reniformis* was exposed to the MTT test using various solvent fractions of the total plant. Human cervical cancer Hela and human breast carcinoma MCF cell lines were discovered to be cytotoxic by the ethylacetate fraction of the whole plant. The ethylacetate fraction's IC_{50} value was 51.57 g/ml for Hela cell lines and 39.6 g/ml for MCF-7 cell lines. Significant outcomes were seen, consequently supporting the use of plants in the conventional medical system. Along with a well-known Coumarin derivative called Scopoletin, the irodoids gardenoside were also isolated for the first time from an *Ipomoea* species, specifically *Ipomoea reniformis* (Convolvulaceae). Its spectroscopic measurements were used to establish its structure.

Keywords: Anticancer activity, MTT assay. Scopoletin, UV spectroscopy, IR Spectroscopy, NMR spectroscopy, Mass Spectroscopy.

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INTRODUCTION

The perennial herb (creeper) *Ipomoea reniformis* belongs to the family Convolvulaceae. It is widely spread over all of India, notably in wet areas of the upper Ganges plain, Gujarat, Chhattisgarh, Bihar, West Bengal, Western Ghats, ascents to 900m in the highlands, Goa, Karnataka, Ceylon, and Tropical Africa[1-7,9]. The synonym of *Ipomoea reniformis* is *Merremia emarginata* Hallier. in India, it is known by many names in different regions, including Mooshakarni in Sanskrit, Underkani in Marathi, Indurkani in Bengal, Underakani in Gujarat, Toinnuatali in Telugu, Chukakani in Urdu, Goromusha in Persian, Mushkani in Hindi, Paerattaekirae in Tamil, and Yellikkadukirai in Madras [2,5,7-10]. There are numerous significant claimed therapeutic benefits of it. In the Native *Ipomoea reniformis*, according to the Traditional Chinese Medicine (TCM) system of medicine, is beneficial for renal disease, fever caused by liver enlargement, cough, headache, neuralgia, rheumatism, diuretic, inflammation, and nose problems. The root contains a diuretic and a laxative and is used to treat eye illness and gums, while the powder from the leaves is used as a snuff during epileptic episodes. For its therapeutic uses, the entire plant decoction is primarily to blame [7–11]. The plant reportedly includes resin and glycosides, according to investigations in the literature. Amino acids, tannins (condensed tannins, pseudo tannins), and caffeic, pcoumaric, ferulic, and sinapic acid esters[12].

More than one-third of the world's population suffers from cancer, which accounts for more than 20% of all fatalities and is a leading cause of mortality. Tobacco, viruses, chemicals, radiation, environmental variables, and nutritional factors are a few of the factors that might cause cancer[13]. The primary conventional cancer treatments in China include chemotherapy and radiation, which are frequently complemented by other complementary and alternative therapies[14]. In the past, people have treated cancer with plants as a natural cure. Etoposide and Teniposide, clinically effective medications used to treat lymphomas, bronchial cancer, and testicular cancer, were developed as a result of extensive research conducted at Sandoz facilities in Switzerland in the 1960s and 1970s[15]. By restoring physiological homeostasis and training the body tissues, these plants may support the host's resistance to infection. According to numerous research, medicinal plants' ability to fight cancer is a result of the antioxidants that are naturally present in them. Compared to contemporary (allopathic) medications, therapeutic plants are more readily available, less expensive, and toxic-free[16]. The creation of new plant-derived natural compounds and their analogues for anticancer activity describes attempts to synthesis novel derivatives based on bioactivity- and mechanism of action-directed isolation and characterization coupled with rational drug design-based modification[17]. Cellular communication with the outer world is regulated by oncogenes. Proto-oncogenes are mutated to produce them. Exposure to chemical, environmental, or viral carcinogens stimulates mutated oncogenes, which causes cell changes and causes them to

produce proteins that are either inappropriately expressed in their normal cell or inappropriately expressed in other tissues, which causes cellular proliferation and, ultimately, the development of cancer. Tumour suppressor genes are designed to inhibit oncogenes by preventing unchecked cellular proliferation. Tumour suppressor genes oppose oncogenes, which cause cancer when active or amplified and work to prevent cancer when inactivated or suppressed. Two of the most prevalent tumour suppressor genes involved in the generation of cancer are p53 and retinoblastoma or Rb[18].

MEDICINAL PLANTS

***Aegle marmelos* Correa ex Roxb.(Bel; Family: Rutaceae)**

A. marmelos has substantial antioxidant activity, lowers the negative effects of chemotherapy and radiotherapy, and has strong anticancer action against breast cancer, malignant lymphoma, malignant melanoma, malignant ascites, and leukaemia.

***Allium cepa* Linn (piyaz/onion; Liliaceae/Alliaceae family)**

Diallyl disulphide, allicin, allin, and vitamins (C, E), extracted from the bulb of A. cepa, detoxify carcinogen, inhibit Helicobacter pylori and halt cell cycle from S to G2M phase. In addition to quercetin's potential to treat lung and other cancers, diallyl disulphide also prevents stomach cancer[21].

***Allium sativum* Linn. (Lasun/garlic; Liliaceae/Alliaceae family)**

From the bulb of A. sativum, sulphur compounds (diallyl sulphide, diallyl disulphide, and allyl propyl disulphide) and allicin have been identified. Cancer cell development is inhibited by allicin and sulphur compounds, respectively, in the stomach, liver, colon, breast, and endometrium[21].

***Aloe vera* Tourn. ex Linn./*A. barbadensis* Mill. Indian aloe (Ghee-Kunwar; Family: Liliaceae)**

Acemannan, a polysaccharide that may be extracted from the root, pulp, leaves, or aerial parts of *A. vera*, has strong anticancer action and boosts the immune system. Strong anti-cancer and immune-boosting properties are displayed by the lectins and emodin that were extracted from this herb. Aloe-emodin triggers apoptosis, which prevents stomach cancer and other sarcomas from growing and spreading. About neuroectodermal malignancies, aloe-emodin displays selective anticancer action. Strong anticancer efficacy against leukaemia is shown in the *A. vera* isolate alexin B. Its polysaccharides possess potent immunostimulating and anticancer qualities. In particular, liver cancer is protected from "super carbohydrates" found in *A. vera*. This plant stops the development of cancer, slows its growth, and stops it from spreading. By triggering macrophages to release cytokines including interferon, interleukin, and tumour necrosis factor, *A. vera* increases the body's immunological response. The negative effects of radiotherapy and chemotherapy are diminished by *A. vera* exceptional antioxidant profile. Its leaves contain glycosides-hydroxyanthraquinone derivatives or anthracene derivatives[20].

***Andrographis paniculata* Wall. ex Nees (Kiryat/Kalmegh/Creat; Family: Acanthaceae)**

Andrographolide, an active diterpene component isolated from the entire plant of *A. paniculata*, shows immune stimulatory and potent anticancer effects on malignancies of the breast, ovary, stomach, colon, prostate, kidney, and nasopharynx, as well as malignant melanoma and leukaemia. The generation of white blood cells, which serve as our body's defence cells, the release of interferon, an antiviral factor, and the activity of the lymphatic system, the centre of our immune system, have all been shown to be potent immune system enhancements that andrographolide may improve. By stopping the cell cycle in the G0/G1 phase and triggering apoptosis, andrographolide has a direct anticancer effect against cancer cells. There is significant anticancer efficacy against colon cancer in the dichloromethane fraction of the methanolic extract of *A. paniculata*. Human epidermoid carcinoma of the skin, nasopharynx lining, and lymphocytic leukaemia cells have all been shown to be cytotoxic (cell-killing) against cancer cells by *A. paniculata* extract. In mice, the anti-chemotoxic and anti-carcinogenic properties of *A. paniculata* were seen. As a result, *A. paniculata* has anti-cancer, immune stimulant, antioxidant, anti-HIV, anti-inflammatory, and anti-hepatotoxic effects. Additionally, it lessens the negative effects of radiotherapy and chemotherapy by enhancing the activity of protective liver enzymes. Flavonoids and andrographin are other components of *A. paniculata*[22].

***Azadirachta indica* A. Juss./*Melia azadirachta* Linn. (Neem; family: Meliaceae) A. Juss./*Melia azadirachta* Linn.**

About 40 distinct active substances, known as limonoids, are found in the stem bark, leaves, and flowers of *A. indica*. These limonoids have anti-inflammatory, anti-cancer, anti-metastatic, anti-ulcer, antifungal, and antiviral properties in addition to immunostimulating and antioxidant properties. Multiple malignancies, including those of the breast, lung, liver, stomach, prostate, and skin, are inhibited in their growth and spread by limonoids. By inducing apoptosis (programmed cell death), a process that instructs the body's immune cells to recognise and eliminate cancer cells, nimbolide, a natural triterpenoid isolated from *A. indica* leaves and flowers, inhibits the growth and spread of a variety of cancers, including colon cancer, malignant lymphoma, malignant melanoma, and leukaemia. Furthermore, nimbolid stops cancer from spreading. By triggering apoptosis and having an antiandrogenic activity, the ethanolic extract of *A. indica* slows the growth and spread of prostate cancer. Chemotherapy and radiation therapy have less negative effects when this herb is used. Dexamethasone, tannin, βsitosterol, Nimbin, quercetin, and carotene are other polyphenolic compounds found in *A. indica*[19,22].

***Curcuma longa* Linn./C. domestica Valeton (Haldi/Turmeric; Family: Zingiberaceae)**

Initiation, growth, and metastasis of cancer are all suppressed by the curcumin (Diferuloyl methane) and curcuminoids found in the rhizome (tuber) of *Curcuma longa*. The pigment in haldi that gives it its colour, curcumin, causes apoptosis (programmed cell death) and stops the growth of cancer cells in the G2/S phase. An anti-inflammatory, anti-tumour, and antioxidant profile of curcumin has been established. At a critical stage in the development and metastasis of cancer, angiogenesis is inhibited. To stop the growth and spread of oestrogen-positive breast cancer, genistein (derived from Glycine max) and curcumin work in concert. Even in breast tumours that are resistant to multiple medications, turmeric works. By inhibiting cancer cell adhesion, curcumin stops the spread of cancer cells. It prevents the growth and spread of several types of cancer, including those of the breast, lung, oesophagus, liver, colon, prostate, head, neck, and skin Radiotherapy-resistant prostate cancer responds well to curcumin in particular. Even in advanced cancer stages, it is still beneficial. In male albino Wistar rats, curcumin had a chemopreventive impact on the hepatocarcinogenesis that was brought on by N-nitroso diethylamine and phenobarbital. Furthermore, it safeguards against colon and stomach cancer. Furthermore, the rhizome of *C. longa* has antimutagenic, antioxidant, immunostimulant, anti-inflammatory, radioprotective, stimulant, alterative, blood purifier, hepatoprotective, antiperiodic, and tonic properties. Rhizomes are also efficient in treating solid tumours such as stomach papillomas, leukaemia, hepatocellular carcinoma, fibrosarcoma, bladder, prostate, and colon malignancies, as well as intravesical tumours, fibrosarcoma, and hepatocellular carcinoma[19,23].

***Phyllanthus emblica* Linn./*Emblica officinalis* Gaertn. (Amla/Amlika/Indian Gooseberry; Family: Euphorbiaceae)**

Flavonoids, glycosides, proanthocyanidins, quercetin, kaempferol, emblicanin, gallic acid, and ellagic acid are all found in *E. officinalis* fruit. It is prized for its distinctive tannins and flavonoids, which have strong immunomodulatory, antioxidant, and anticancer effects. Ellagic acid is an effective antioxidant with the capacity to prevent gene alterations. Chromosome abnormalities are also fixed by ellagic acid. Hepatoprotective effects are provided by quercetin. Strong antioxidant and anticancer effects can be found in the tannins emblicanins A and B. Different malignancies, such as those of the breast, uterus, pancreas, stomach, and liver, as well as malignant ascites, are prevented from growing and spreading by *E. officinalis*. The herb *E. officinalis* works wonders for antioxidants and skin renewal. It is very nutrient-dense and a significant source of phyllembic acid, lipid, emblicol, colloidal complexes, mucic acid, amino acids, and minerals. Vitamin C is also a potent antioxidant. Many cancers are protected from *E. officinalis*, but liver cancer is one in particular. Chemotherapy and radiation therapy's negative effects are lessened. Astringent, diuretic, cooling, aperient, refrigerant, acrid, and laxative are other qualities of amla fruits. Inflammation, bleeding, cough, diarrhoea, anaemia, jaundice, and dyspepsia are among the conditions that the dried fruits are helpful for. In contrast to the root and bark, which are astringent, *E. officinalis* flowers are cooling, refrigerant, and aperient. Biliousness, bronchitis, and asthma are treated using *E. officinalis* seeds. Amla, a fruit high in vitamin C (ascorbic acid or ascorbate), is used to treat human scurvy. Leucodelphinidin, phyllembin, tannin from fruit, bark, and leaves, fixed oil, essential oil, and phosphatides from seeds were all found in the bark of *E. officinalis*. Phyllembin was discovered in fruit pulp and was later identified as ethyl gallate. 18 chemicals in the amla fruit inhibit the growth of breast, uterine, and stomach cancers. Natural killer cell activity is increased in a wide variety of malignancies. The number of Dalton's lymphoma ascites cells that produce ascites and solid tumours in mice was decreased by its extract. Animals with tumours lived longer when the extract was administered to them[19,24].

LITERATURE METHODS

Extraction/fraction of plant material

The plant material was taken in the Tirunelveli area of Tamilnadu, India, in March 2011. Prof. Jayaraman of the Plant Anatomy Research Centre in Tambaram, Chennai, Tamilnadu, India, botanically recognised and verified the plant material. *Ipomoea reniformis* complete, shade-dried plant was ground into a coarse powder. until complete extraction has been achieved using ethanol in soxhlet extraction equipment. Column chromatography was used to separate the solvent-free fraction, which was then further fraction by successive solvents using hexane, chloroform, ethyl acetate, and methanol solvent. The MTT cell proliferation assay was then performed on each of these fractions[25].

MTT CELL Proliferation Assay

Cell Type and Culture

MCF-7 (human breast carcinoma) and Hela (human cervical carcinoma) cell lines were obtained from the National Centre for Cell Science in Pune, India. Penicillin (100 units/ml) and streptomycin sulphate (100 g/ml) antibiotics were added to the growth medium (DMEM, PH-7.4), which was used to cultivate the cells[26,27].

MTT assay

The cells were seeded into the wells of a 96-well microtitre plate (Costar 3599, Corning, NY, USA) at a density of 2 x 104 cells per well with 100 µl of DMEM growth media. The cells were then incubated for 24 hours at 37°C with 5% CO2 in a humid environment. The medium was then taken out and a new growth media was introduced, including various test doses at 100, 50, 25, 12.5, 6.25, and 3.125 g/ml. The medium was taken out and each well was given 100 µl of DMSO before being gently shaken after three days of incubation at 37 °C with 5% CO2. ELISA reader (Biorad,

Hercules, California, USA), which measures absorbance at 490 nm, was then used to calculate it. Only the medium was administered to control wells; no test sample was included. The study's positive control was the traditional anticancer medication 5-fluorouracil[28]. Using the methodology below, the per cent anticancer activity was estimated as the suppression of cell growth.

$$\text{Cells inhibition} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Analysis of statistics

Data were reported as Means Standard Deviations of three replicate determinations, and then SPSS v.13 one-way analysis of variance (ANOVA) and Dancan's new multiple range test were used to identify differences between the means. Significant P values were considered to be those below 0.05[29].

Screening Procedures for Phytochemicals [30,31]

To test for the presence of alkaloids, carbohydrates, and reducing sugars, all the crude extracts underwent preliminary phytochemical screening using industry standard procedures.

Glycosides, proteins and amino acids, steroids and triterpenoids, phenolic substances and tannins, flavonoids, fixed oils and fats, volatile oils, gums, and mucin. Below is a description of typical practices.

A. Carbohydrate test

Test for Molisch: After adding concentrated H_2SO_4 from the test tube's sidewalls to the 2–3 ml test solution, a few drops of the alpha–naphthol solution in alcohol are added.

Fehling's test: 1 ml of Fehling's A, B, and test solutions were combined, and they were heated for 1 minute in a boiling water bath before being added. This process took 5 to 10 minutes.

For gums: Using diluted HCL to hydrolyze the test material, do Fehling's or Benedict's test, where a red colour is formed.

For mucilage: Ruthenium red solution is used to test the drug's powdered form.

B. Protein test

Biuret test: 4% NaOH and 1%$CuSO_4$ are added to 3 ml of the test solution, respectively.

C. Examine the amino acids

Ninhydrin test: 5 drops of Ninhydrin Reagent were added to 1 ml of the test solution, and the mixture was heated in a water bath at a rolling boil for 10 minutes.

D. Glycoside screening: A few drops of ferric chloride and strong sulphuric acid are added to the extract's glacial acetic acid solution, and the upper layer's bluish-green colour and reddish-brown colouring are then checked for.

Legal's test: which detects cardiac glycosides: Afterward, 1 ml of sodium nitroprusside solution is mixed, and 1 ml of pyridine is added to the test solution.

Foam test: for the detection of saponin glycosides Be sure to give the dry powder or medication extract a good shake. use water for.

E . Organic acid testing

Malic acid screening: 2-3 ml of Test solution 5% $FeCl_3$ solution is added in two to three drops.

Tartaric acid screening: A 3ml test solution is added. The solution was heated for a few minutes after 2 drops of a 2% resorcinol solution and 3 ml of Con H_2SO_4 were added.

F. For vitamin C: 2ml of a 2% w/v solution and 20 g of ferrous sulphate is combined with 2 ml of water, 0.1 g of $NaHCO_3$, and then shaken. The mixture is then left to stand.

G. Test for flavonoids

Shonda test: A few drops of strong HCl are also added to the test solution, along with 5 ml of 95% ethanol, and then 0.5 g of magnesium turnings.

H. Alkaloids Test

Test for Dragendorff's Reagent: A few drops of the reagent were added to 2-3 ml of the test solution.

Test for Wagner's: Add a few drops of Wagner's to 2–3 ml of filtrate.

I. Libermann-Burchard test, detects phytosterols: In 2 ml of acetic anhydride, 50 mg of the extract is dissolved. This is then given a gentle addition of 1 or 2 drops of concentrated sulfuric acid down the test tube's sides. Phytosterols are manifested through a variety of colour changes.

J. For phenolic chemicals

- ✓ 2ml of extract is treated with a few drops of 5% of ferric chloride solution
- ✓ 2ml of the extract is treated with a few drops of lead acetate solution
- ✓ 2ml of extract is treated with a few drops of gelatine solution
- ✓ 2ml of extract is treated with a few drops of bromine water solution
- ✓ 2ml of the extract is treated with a few drops of acetic acid solution
- ✓ 2ml of extract is treated with a few drops of potassium dichromate
- ✓ 2ml of the extract is treated with a few drops of dilute iodine solution.
- ✓ 2ml of the extract is treated with a few drops of nitric acid solution.

- ✓ 2ml of extract is treated with a few drops of ammonium hydroxide solution, potassium and ferric cyanide.
- ✓ 2ml of the extract is treated with one drop of ammonium hydroxide solution, excess of 10% silver nitrate, and heat for 20 minutes in boiling water bath.
- ✓ 2ml of the extract is treated with a few drops of dilute potassium permanganate solution Isolation and withdrawal.

Sampling process preparation[32]

In preparation for thin-layer chromatography, the methanolic extract was concentrated. Preparative thin-layer chromatography involves detecting the substance of interest, removing it from the layer, and then analyzing it using the appropriate analytical technique. Toluene, ethyl acetate, and formic acid (5:4:1) were the mobile phase that was employed. The plate was spotted, developed in a solvent system, dried, and then examined in a UV cabinet under 366nm UV light. It was possible to see four locations with Rf values of 0.47, 0.6, 0.88, and 0.95. Methanol was added, filtered, and added to one area with an Rf value of 0.47. One sample, isolated chemical V (R: 0.47), perhaps scopoletin, was obtained after the filtrate was evaporated. To validate and expand upon the sample's identity, TLC, chemical tests, and spectroscopic examinations were then performed on it.

RESULTS PUBLISHED

Phytochemical screening

The results of the preliminary phytochemical analysis, which was conducted to identify the major ingredients found in the extract, are shown in Table 1.

Table 1: Provides the specifics.

Name of constituent	Petroleum ether	n-hexane	Chloroform	Ethyl acetate
Carbohydrate	--	--	--	--
Proteins	--	--	--	--
Amino acid	--	--	--	--
Glycosides	--	--	+	+
Malic acid	--	--	--	+
Vitamin c	--	--	--	--
Flavonoids	--	--	+	+
Alkaloids	--	--	--	+
Phytosterols	--	--	+	--
Phenolic	--	--	+	+

Table 2: The IC_{50} values of *Ipomoea reniformis* against human cervical carcinoma Hela and human breast carcinoma MCF-7 cell lines.

Different fractions of *Ipomoea reniformis*	Cytotoxicity IC_{50} values (µg/ml)	
	Hela	MCF
Hexane (HME)	418.3	434.5
Chloroform (CME)	235.8	211.7
Ethyl acetate (EAME)	51.57	39.6
Methanol (MME)	71.5	46.3
5-fluorouracil	29.6	15.3

MTT assay

The reduction of MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) to purple formazan product by mitochondrial dehydrogenase provides the basis for the MTT assay. The findings of the MTT cell proliferation experiment were performed on the various solvent fractions of the entire *Ipomoea reniformis* plant. *Ipomoea reniformis* was discovered to have cytotoxic activity in its various sections, but only the ethylacetate fraction with an IC_{50} value lower than 200 g/ml was deemed to be active The IC_{50} value for the remaining fractions was looked at, and it revealed that anything beyond 200 g/ml was deemed inactive.

An isolated chemical's evaluation and confirmation

Compound V (R: 0.47) or scopoletin was isolated using preparative thin-layer chromatography with Silica Gel G as an adsorbent. The solvent system used was a mixture of toluene, ethyl acetate, and formic acid (5:4:1).

Microscopy research

Spectroscopy in the UV-VIS range

When the isolated compound V (R: 0.47) and Standard Scopoletin were dissolved in methanol with 2-4 drops of 2M NaOH added.

Characteristic UV bands may be found at 338.12 nm, 296.52 nm, 226.90 nm, and 339.55 nm, respectively, in the UV spectra of Standard Scopoletin in methanolic solution and Isolated Compound V. As a result, it may be deduced that Scopoletin is Isolated Compound V.

The bathochromic shift was seen after 2-4 drops of 2M NaOH were added to a methanolic solution containing both Standard Scopoletin and Isolated Compound V, further confirming that the latter is Scopoletin.

Infrared spectroscopy

The broadband in the peak at 3337.44 and 3341.44 cm-1 of the IR spectral analysis is most likely caused by the phenol OH group's O-H stretching vibrations. The peak at 2850.97 and 2875.05 cm^{-1} displayed C-H Stretching caused by -CH3. The presence of the -C=O, Carbonyl group is shown by the peaks at 1702.90 and 1703.42. The peak at 1628.09 and 1606.75 indicated the presence of the -CH=CH group. The existence of a benzene ring is shown by the peak at 1565.06, 1510.53 & 1568.83, 1511.16. The peak at 861.46 & 861.50 revealed the existence of benzene ring disubstitution in both isolated compound V and normal Scopoletin, respectively. The comparison with the standard mentioned above demonstrates that isolated chemical V is Scopoletin (Figures 1 and 2).

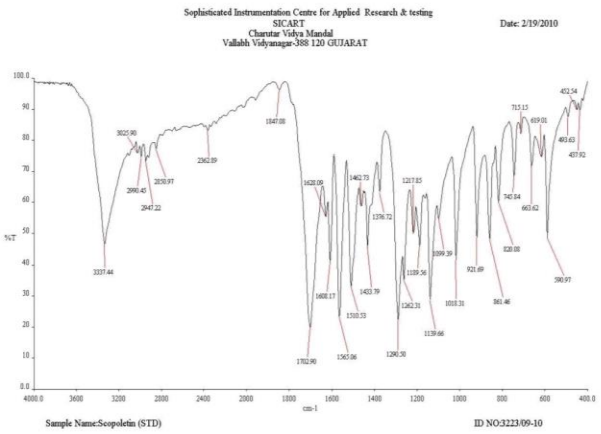

Figure 1: The IR spectrum of standard Scopoletin.

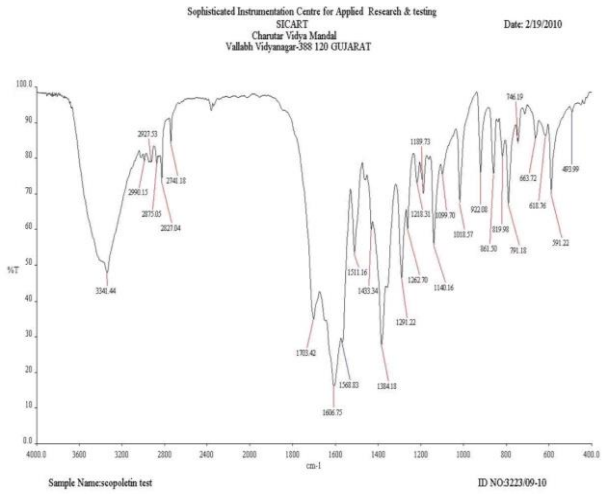

Figure 2: The IR spectrum of Isolated Compound V.

NMR spectrography

The ^{1}H NMR spectra of standard Scopoletin and Isolated Compound V revealed two doublets with coupling constants of 9.2 Hz at 6.23, 6.22, and 7.88 ppm, which were identified as H- 3 and H-4, respectively, in standard Scopoletin and isolated compound the coumarin-specific compound V.

Scopoletin's 1H NMR spectra revealed two aromatic singlets at 7.14, 7.13, and 6.79 ppm and a methoxyl group singlet at 3.93 ppm in both the standard form and the isolated form, respectively. The 6,7-disubstitution was used to explain compound V. Therefore, it is proven that isolated chemical V is scopoletin.

Mass spectrography

Standard Scopoletin and isolated Compound V's mass spectra both exhibit the M-1 peak at 191.10, indicating that their molecular weights are both 192.10, according to their respective mass spectra. Therefore, it is confirmed that Scopoletin (Figures 3 and 4).

Figure 3: Mass spectrum of standard Scopoletin.

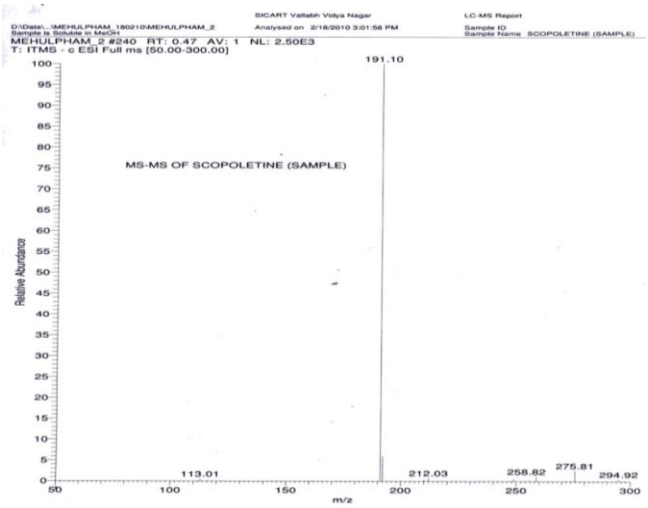

Figure 4: Mass spectrum of Isolated Compound V.

Melting point

The results from standard and isolated compound V's melting points are 207 and 206.2 respectively. Isolated Compound V's and standard scopoletin's melting points are identical.

CONCLUSION

The primary topics covered in the review include the medicinal plant used for anticancer activities, Components and Methods that include Extraction/fraction of plant material, MTT CELL PROLIFERATION ASSAY that includes

various tests like Cell type and Culture, MTT assay, Analysis of Statistics, Screening Procedures for Phytochemicals, Sampling Process Preparation that further contains various tests like IR Spectroscopy, NMR spectroscopy, Mass Speactrography, etc.

The purpose of this review's representative was to show how medicinal herbal extractions are used to fulfil beneficial needs, with a focus on how cancer is the primary cause of people who have incurable diseases because of the widespread use of antibiotics and dosage regimens that, inadvertently, damage other body parts. As a result, the cancer goes untreated or gets worse.

Additionally, the primary method of treating cancer that has been examined thus far in numerous research projects involves increasing immunity so that the body's immune cells receive the nutrients they need and can fight off malignant cells.

Avoiding allopathic therapy to prevent the unintended and unanticipated damage that excessive use of allopathic supplements causes to the body. Herbal medicine has seen a significant change in usage and is effective by several studies and reviews.

The numerous tests carried out to determine its effectiveness and quality have demonstrated that it is very effective, less harmful, and more affordable than other allopathic treatments.

Thus, the data of many carcinogenic medical treatments using herbal plants that have been mentioned in this article's discussion of cancer's global causes offer us the facts and results that also clarify the legitimacy of using herbal medicines to cure cancerous cells.

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REFERENCES

1) Kirtikar KR, Basu BD, Indian medicinal plants, 2nd ed., Vol. 2, Lalit Mohan basu, Allahabad, India, 1702 (1935).

2) Shah GL, Flora of Gujarat State 1st ed., Vol. 1, Sardar Patel University, Vallabhvidyanagar, 459-460 (1978).

3) Ansari SH, Essential of Pharmacognosy, 5th ed., Birla publication, page no. 408-410 (2005-2006).

4) Agarwal VS, Drug plants of India, 1st ed., Vol. 2, Kalyani printings, New Delhi, 499 (1997).

5) Satyvati VG, Gupta K. A., Medicinal Plants of India 1st ed., Vol. 2, Indian Council of Medical Research, New Delhi, 241 (1987).

6) Backer CA, Flora of Java, 576-578.

7) Chatterjee A, The Treatise of Indian medicinal plants, 1st ed., Vol. 4, National Institute of Science Communication And Information Resources, New- Delhi, 148-149 (2003).

8) Agarwal VS, Drug Plants of India, 1st ed., Vol. 1, Kalyani Publishers, New Delhi, 440 (1947).

9) Warden HJ, Hooper D, PharmacographiaIndica, Vol. 2, Bishen Singh, Delhi, 539 (1890).

10) Dutta AC, Botany, 6th ed., By Oxford University Press, Calcutta, 576-577 (2000).

11) Shah CS, Sukkawala VM, Chavan AR, Pharmacognostic Study of *Ipomoea reniformis* and Comparision With CentellaAsiatica Linn, 22 (1962).

12) Rastogi PR, Mehrotra BN, Compendium of Indian Medicinal Plants, 1st ed., Vol. 2, Published By Indian Council of Medical Research, New Delhi, 241 (1993).

13) Lemkebthomas L., Williams D. A., Roche V. F., William Z. S., Foye's principles of medicinal chemistry., 6th edition, 2008, 1147-1148

14) Z., Michael S., Eran Ben-A., and Bashar S., Greco-Arab and Islamic Herbal-Derived Anticancer Modalities: From Tradition to Molecular Mechanisms, Evidence-Based Complementary and Alternative Medicine., 2012,13

15) Wen T., Jinjian L., Mingqing H., Yingbo Li., Meiwan C., Guosheng W., Jian G., Zhangfeng Z., Zengtao X., Yuanye D., Jiajie G., Xiuping C., and Yitao W., Anti-cancer natural products isolated from Chinese medicinal herbs, Chin Med., 2011, 6, 27.

16) Prema R., Sekar S.D., Chandra Sekhar K B., Review On: Herbs As Anticancer Agents, Int. J. Pharma & Ind. Res., 2011;11:105-109.

17) Pandey G and Madhuri S., Some medicinal plants as natural anticancer agents, Phcog Rev., 2009;3:259-263.

18) Dholwani K.K., Saluja A.K., Gupta A.R., and D.R. Shah., A review on plant-derived natural products and their analogues with anti-tumour activity, Indian J Pharmacol., 2008;40:49–58.

19) Pandey Govind, Madhuri S. Some anticancer agents from plant origin. Pl Arch 2008; 8(2): 527-532.

20) Madhuri S, Pandey Govind. Ethnomedicinal plants for prevention and treatment of tumours. Int J Green Pharm 2009; 3(1):2-5.

21) Pandey Govind. Antioxidant vegetables act against cancer and other diseases. Int J Pharmaceu Stud Res 2011; 2(1):32-38.

22) Pandey Govind, Madhuri S. Some medicinal plants as natural anticancer agents. PHCOG REV 2009; 3(6):259-63.

23) Pandey Govind. Active principles and median lethal dose of Curcuma longa Linn. Int Res J Pharm 2011; 2(5):239-241.

24) Pandey Govind, Pandey SP. Phytochemical and toxicity study of Emblica officinalis (Amla). Int Res J Pharm 2011; 2(3):270-272.

25) Dominic Dufour. Antioxidant, anti-inflammatory and anticancer activities of methanolic fractions from *Ledum groenlandicum* Retzius, *Journal of Ethnopharmacology,* 2007;111: 22-28.

26) The ayurvedic pharmacopoeia of India,(1999), Part-I, Vol-II, The controller of the publisher, 69-70.

27) Nadkarni.K, (1988) *Indian Materia Medica*, Bombay Popular Prakashan, 858-860.

28) Rana Abu-Dahab and Fatma Afifi, Anticancer activity of selected medicinal Plants of Jordan against a breast adenocarcinoma cell line (MCF-7), *Scientia Pharmaceutical*, 2007;75:121-136.

29) Kulkarni. S.K, Handbook of Experimental Pharmacology, Vallabh Prakashan, 2007;172-189.

30) William Charles Evans "Trease and Evans Pharmacognosy", 16th edition, pp.196(119).

31) Khandelwal K. R., "*Practical Pharmacognosy techniques and experiments*", 7th edition, pp.154, 2007.

32) Bhatt Mehul K, Dholvani Kishor K, Isolation and Structure Elucidation of Scopoletin from *Ipomoea reniformis* (convolvulaceae), Journal of Applied Pharmaceutical Science 01(05); 2011: 138-144.

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