**In-vitro ANTICANCER ACTIVITY OF Saurauia vulcani LEAF EXTRACTS AGAINST HUMAN COLON WIDR AND HCT-116 CANCER CELLS**

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**ABSTRACT**

*Saurauia vulcani* plants grow naturally in North Sumatra, Indonesia. This plant is commonly used as an antidiabetic drug and a cure for other digestive ailments. The objective of this study was to examine the phytochemical characteristics and anti-colorectal-cancer activity of the extract from leaves of *Saurauia vulcani* plants. The method employed was to test phytochemicals that included the presence/detection of alkaloids, saponins, flavonoids, triterpenoids/steroids, tannins, and hydroquinone in the leaf extract. The anti-colorectal-cancer activity was tested in vitro using the MTT method, against WiDr and HCT 116 cancer cells, with the liquid fractions of n-hexane, ethyl acetate, and methanol solvents. Phytochemical analysis results showed that *Saurauia vulcani* leaf extract contained tannins, saponins, flavonoids, and terpenoids. Chemical component analysis of ethyl acetate fraction showed the presence of fatty acid groups. The n-hexane, ethyl acetate, and methanol fractions exhibited cytotoxicity against the WiDr cell line, with IC\(_{50}\) values of 456.19, 97.41 and 191.92 ppm, respectively. Meanwhile, the cytotoxicity activity of the leaf extract against the HCT cell lines was accomplished at IC\(_{50}\) values of 777.35, 568.53 and 529.39 ppm, correspondingly with n-hexane, ethyl acetate and methanol fractions, respectively. The leaf extract in ethyl acetate fraction was indicatively the best in efficaciously inhibiting the WiDr cells.

**Keywords:** *Saurauia vulcani*, Plant, Leaf Extract, Colorectal-cancer Cells, Anti-cancer Activity

**INTRODUCTION**

*Saurauia vulcani* plant has been used traditionally by people in North Sumatra as herbal medicine. As such, the leaves (mainly; Fig. 1) and other portions of the plants (e.g., branches, twigs, bark, and roots) can be used as an anti-diabetic drug and as a remedy to cure other diseases related to the digestive system. This plant is classified into a particular group of trees (that could be under the same genus/species), which belongs to the family Actinidiaceae. Generally, *Saurauia vulcani* (with its vernacular name as “pirdol”) grows wild at the edges of forests. This plant can be easily be found along the Sumatra highway starting from the districts of Simalungun, Toba, and North Tapanuli all the way elsewhere near the highway.\(^1\)

Research on *Saurauia vulcani* plants and their groups in the same genus includes possible disease remedies or medicinal actions as antioxidants, anti-cholesterol, antibacterial, and immunomodulators. This plant, according to the phytochemical information, contains specific chemical compounds such as alkaloids, flavonoids, terpenoids, glycosides, saponin and tannins.\(^2,3\) On the other hand, unfortunately, research regarding the possible anticancer actions of this plant so far has never been carried out.

Several research results have disclosed that certain portions of the plants (e.g., stems, branches, twigs, leaves, roots, seeds, and fruits) contain specific chemical compounds, such as paclitaxel, etoposide, camptothecin, vinblastine, vincristine, topotecan, and irinotecan, which indicatively afford potencies as anticancer actions. Further, groups of fruits and vegetables could perform such remarkable metabolic processes that they produce specific chemicals, among others curcumin, lycopene, saponin, isoflavone, cucurbitacin, sitosterol, and resveratrol, indicatively efficacious as anticancer agents.\(^4\) Research on anticancer agents derived from natural products for cancer remedies (especially from natural renewable
sources) is continuously and intensively carried out throughout various parts of the world. As well as Syzygium aqueum stem bark has anticancer activity against cervical and breast cancer.\textsuperscript{5} Such thorough and rigorous undertaking is due mainly to the increasing prevalence of cancer-afflicted patients, especially colorectal-cancer diseases. Currently, the colorectal-cancer diseases have ranked the third that leads to cause enormous human death in cancer patients, after breast and ovarian cancer diseases as the first and second ranks, respectively.\textsuperscript{6}

Malaysia is a neighboring country that has almost similar physical and natural environments. As such, several types of medicinal plants from Malaysia have been tested to cure colorectal-cancer disease, including Annona muricata, Baccaurea motleyana, Casearia capitellata, and Curcuma manga, Garcinia mangostana, Pereskiea ble, Phyllanthus pulcher, Strobilanthus crispus, and Zingiber officinale.\textsuperscript{8}

Colorectal cancer signifies a type of cancer commonly experienced by or suffered by people throughout various parts of the world. This cancer disease becomes the third most common kind of cancer that could occur in the bodies of men and women. An unhealthy lifestyle, poor diet, smoking, colitis, genetic factors, and the aging process could contribute to the causes of colorectal cancer disease.\textsuperscript{7,8}

Research on renewable natural ingredients (e.g., plants and other living creatures) as a treatment for colorectal cancer disease has been widely reported using so-called Chinese Herbal Medicines (CHMs). The mechanism of CHMs is such that they can modulate proliferation, apoptosis, cell cycle, adhesion, migration, and angiogenesis in colorectal cancer cells. Several species of plants studied include Curcuma longa, Ginkgo biloba, Pogostemom cablin, Andrographis paniculate, and Artemisia sp.\textsuperscript{9}

Conventional cancer treatment with chemotherapy could occasionally bring out unexpected results, whereby it causes drug resistance and side effects to cancer patients. Various attempts were made to reduce these side effects. They combine chemotherapy colorectal cancer treatment with renewable natural ingredients (e.g., plants and other living creatures). Relevantly, the purpose of this study was to examine the anti-colorectal-cancer activity of Saurauia vulcani extract using two cancer cells, which comprised WiDr and HCT 116.

Further, research on renewable natural products, among others those extracted from Saurauia vulcani plants, as the anti-colorectal-cancer agent has unfortunately never yet been done before. Meanwhile, initial signals of phytochemical information obtained from the genus Saurauia indicate that it contains specific chemical compounds (e.g., flavonoid, tannins, and terpenoids). These compounds belong to a group of compounds reportedly to have efficacious potencies as anti-colorectal cancer agents.\textsuperscript{10}

**EXPERIMENTAL**

**Material and Methods**

The main materials used in this research/experiment were inherently the Saurauia vulcani leaves (Fig.-1), which have been procured from their host plants/trees. The host (Saurauia vulcani) trees grew widespread and profusely at the Sipiso-piso’s Special Purpose Forest Area, administratively located in the Karo Regency, North Sumatra Province, Indonesia. Meanwhile, the cancer-cell materials consisted of WiDr cells and HCT 116 cells.

**Extraction**

Initially, Saurauia vulcani leaves (about 1 kg in weight) were chopped off to very small pieces and then ground to powder. Afterward, some amount in weight of the resulting Saurauia vulcani leaf powder (with its known equivalent oven-dry weight) was taken and further extracted using a multilevel extraction method for 3-by-24 hours. The leaf extraction was carried out using three kinds of organic solvents, comprising n-hexane, ethyl acetate, and methanol. The obtained leaf-extracted filtrate (with any of the three extracting solvents) was then concentrated using a rotary vacuum evaporator.

**Phytochemical Testing**

Phytochemical testing on the concentrated leaf-extracted filtrate was further performed, which included, among others, the possible presence/detection of alkaloids, saponin, flavonoid, triterpenoid or steroid, tannins, and hydroquinone in the filtrate.\textsuperscript{11}
Chemical Component Analysis
Chemical component analysis was carried out on the most active fraction using Gas Chromatography-Mass Spectra (GCMS), QP 2010 Shimadzu.

Testing on Anti-Colorectal Cancer Activities
Preparing of Growing Media for the Cancer Cells
The D-MEM, as well as RPMI-1640 media in powder shapes, were dissolved in one liter of deionized distilled water and then added with 3.7 grams of NaHCO₃ for D-MEM; and with 2 grams of NaHCO₃ for RPMI. The water-dissolved media were thereafter sieved using a 0.2-μm filter and then added with consecutively Fetal Bovine Serum (FBS) (Hyclone, USA) and antibiotics. Afterward, the sieved media were kept and stored in the refrigerator set at constant temperature (4°C), which further would be used as culturing/growing media for the cancer cells.

Preparation of Cancer Cell
Monolayer cells (i.e., WiDr and HCT 116 cancer cells), which were inherently the cells grown confluent, should undergo subculture treatment in the flask. The subculture-treated media in the cell-containing flask were then discarded, and into the flask was added 10 mL of Phosphate buffer saline (PBS) (Gibco, USA) to clean the flask of any residual media. The PBS was then discarded as well from the cell-containing media. After that, as much as 5 mL of Trypsin (Gibco, USA) with 0.125% concentration was added into the cell-containing flask, and then the flask was incubated at 37°C for 5 minutes in a CO₂ incubator (at 5% concentration). In this way, the cells in the incubator would form a suspension.

Counting the Number of Living Cells
As much as 50 µL of cell solution at a particular concentration (i.e., containing WiDr cells as well as HCT 116 cells) was added with 50 µL of trypsin blue (as coloring agent), then flowed into the hemocytometer device. Afterward, the observation was performed on the cells (WiDr as well as HCT 116 cells), which were still alive or survived (indicated by the cell’s ability to absorb the color). Further, the cells (whether still alive/survived or already dead) in the solution form were removed from the hemocytometer device and then placed into two big boxes. Inside each of the two boxes, the cell solution was added in stages with water (as a diluting liquid). After the dilution, the number of living cells (indicated by their color, after previously absorbing the trypsin blue’s coloring agent) could then be counted using the formula, as follows: The total number of living cells (i.e., WiDr cells as well as HCT 116 cells):

\[ \Sigma \text{Living Cells} / \text{mL} = (\text{Average Number of the Counted Cells}) \times (\text{Dilution Factor}) \times 10^4 \]

MTT Assay
The survived/living, or sustained cells (i.e., WiDr cells and HCT 116 cells) grown inside the T25 flask further underwent the subculture treatment. Afterward, the subculture-treated cells were grown on 96 wells tissue culture plates (as the cell-growing media), with the number of 5000 cells/well; and then incubated (on such media) at a particular concentration for 24 hours at 37°C temperature in the incubator (at 5% CO₂). After the incubation, a bioactive compound (i.e., leaf extract) with a concentration of 100µL/well was added.
to each growing media (that already contained the survived/sustained cells). Separately, the growing media containing the survived/sustained cells (i.e., WiDr cells and HCT 116 cells), but without the addition of bioactive compound (without the leaf-extract treatment), were also prepared as a cell control. The bioactive-added (extract-treated) cell-growing media and the control cell-growing media were then incubated again for 48 years at the same temperature (37°C). Afterwards, the MTT (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) the compound was added to either the bioactive-added (extract-treated) cell-growing media or the control cell-growing media, then incubated for 4 hours at 37°C in the incubator (at 5% CO2). After incubated, the cell supernatant that appeared on the media was discarded, while the formazan crystals were dissolved in 70% ethanol. The reading of optical density (OD) was conducted using a microplate reader at 565 nm wavelength.

**Design of Anti-colorectal-cancer In-vitro Testing**

The in-vitro testing on colorectal cancer cells used WiDr cells and HCT 116 cells. In-vitro testing was carried out using the liquid fraction of n-hexane, ethyl acetate, and methanol as *Saurauia vulcani* leaf extract solvents. There were several manipulations in performing the in-vitro testing on colorectal anti-cancer activities at each liquid fraction, as follows: normal cell (WiDr cells as well as HCT 116 cells), colorectal cancer cells without extracts (as cell control), colorectal cancer cells with n-hexane extracts, colorectal cancer cells with ethyl acetate extracts and colorectal cancer cells with methanol extracts.

**RESULTS AND DISCUSSION**

**Phytochemical Activity**

The phytochemical tests on *Saurauia vulcani* plants, represented by the leaves (Fig.-1), revealed that specific chemical compounds were identified, present, and therefore indicatively contained in the plant bodies, more convincingly in their leaf portion (Fig.-1; Table-1). In general, such phytochemicals disclosed the existence of several compounds, such as tannins, saponins, flavonoids, and terpenoids in *Saurauia vulcani* plants (particularly in their leaf portion). Relevantly, results of the phytochemical testing on the extract (from *Saurauia vulcani* plant leaves, obtained using three kinds of extracting organic solvents) are presented in Table-1.

A group of natural compounds prevalent in biomass plants and commonly used for anti-colorectal cancer included mainly carotenoids, anthocyanins, polysaccharides, alkaloids, polyphenols, terpenoids, and unsaturated fatty acids. Results of phytochemical tests, as described above, hinted at the presence of flavonoids/polyphenols, triterpenoid compounds, where those compounds indicate their efficacious potencies as anti-colorectal cancer agents.

Related studies have shown that alkaloid compounds, polysaccharides, polyphenols, terpenoids, and unsaturated fatty acids in particular plant parts also indicatively exhibited potencies to perform anti-colorectal-cancer activities.

Table-1: Phytochemical Analysis on the Extract from *Saurauia vulcani* Leaves, Regarding the Possible Presence of Their Particular Chemical Compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter (Chemical Compound)</th>
<th>n-hexane Extract</th>
<th>Ethyl Acetate Extract</th>
<th>Methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Steroid/Triterpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tanin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Hydroquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Remarks: + = positively present/identified/detected; – = negatively present/identified/detected

Various studies have been reported that several chemical compounds extracted from renewable natural ingredients have anti-colorectal cancer, including curcumin, resveratrol, Ganoderma lucidum, cannabinoids (cannabidiol), flavonoids (epigallocatechin, genistein, apigenin, chrysin, isoliquiritigenin, kaemferenol, and quinercetinids), betenolin a xanthophyll), Gossypol, Isothiocyanates and indoles, Allyl sulfates, and...
Ginkgo biloba. One of the several ways to combine curcumin with conventional chemotherapy agents, which results in the combined stuff, such as 5-FU, can be effective in chemotherapy treatment for overcoming colon cancer.

**Chemical Components**
The results of the chemical composition analysis test showed that the main compounds of the ethyl acetate fraction i.e., Octadecanoic acid (CAS) Stearic acid 2,3-Dihydro-Benzofuran, Neophytadiene, 9-Octadecenal, (Z)- (CAS) Cis-Octadec-9-Ena, 1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate, Stigmasta-5,22-dien-3-ol, acetate, (3. beta.,22Z)- (CAS) stigmasta-5,22-dien, and Dihydroselarene. It has been reported that fatty acid compounds such as oleanolic acid and β-sitosterol from Syzygium aqueum stem bark extract have good anticancer activity.

**Anticolorectal Cancer Activity**
Cytotoxic activity imparted by the extract (from Saurauia vulcani leaves) on WiDr cells and HCT116 cells is presented in Fig.-2 and 3. Testing results with WiDr cancer cells showed that ethyl acetate’s leaf extract exhibited strong cytotoxic activity against those cancer cells; meanwhile, methanol’s leaf extract and n-hexane’s leaf extract afforded moderate to rather weak cytotoxic activity. Concurrently, results of the cytotoxic test with HCT 116 cells showed that methanol’s; ethyl acetate’s, and n-hexane’s leaf extracts exerted no cytotoxic activity. Microscopic features of WiDr and HCT-116 cells after the treatment (i.e., leaf extracts) are shown in Fig.-4 and Fig.-5.

An extract (or in general chemical compounds) can be said able to impart consecutively very strong cytotoxic activity when the IC$_{50}$ values are below 10 micrograms per milliliters, strong cytotoxic activity if IC$_{50}$ values 10-100 micrograms per milliliters, and moderate cytotoxic activity if IC$_{50}$ value 100-500 micrograms per milliliters. IC$_{50}$ values are defined in this regard as the minimum concentration of leaf extract to inhibit the growth of cancer cells, such that the cell-growth capacity is reduced/ inhibited to 50%.

Based on research results conducted by some enthusiastic scientists, it revealed that the cytotoxic effects of the extract from Vernonia amygdalina Delassay plants were found at various IC$_{50}$ values. As such, the plant extraction was conducted using three kinds of organic solvents, comprising n-hexane, ethyl acetate, and methanol. Further, the IC$_{50}$ values for n-hexane’s, ethyl acetate’s, and methanol’s plant extracts were 7934.963 ± 4154.833 micrograms per milliliters (ppm), 9.086 ±0.431 micrograms per milliliter, and 321.131± 9.902 micrograms per milliliters, respectively. It indicates that the ethyl acetate extract afforded very strong cytotoxic activity; meanwhile, methanol extract exhibited moderate cytotoxic activity; and whereas ultimately, n-hexane extract exerted no cytotoxic activity.
In-vitro ANTICANCER ACTIVITY OF Saurauia vulcani LEAF EXTRACTS

G. Pasaribu et al.

Drug resistance factors and drug effects on colorectal cancer management become the driving-inducement to perform intensive research and development on renewable natural ingredients as anti-colorectal-cancer agents.\textsuperscript{11} One of the medicinal plants, \textit{Sesuvium portulcastrum} diethyl ether extract, showed the highest activity with IC\textsubscript{50} values of 182.86 ± 4.29 μg/mL for HCT-116 cell lines.\textsuperscript{20}
In-vitro ANTICANCER ACTIVITY OF Saurauia vulcani LEAF EXTRACTS

G. Pasaribu et al.

Research on the viability and proliferation of WiDr cells using the MTT method has been carried out using a poly-isoprenoid compound, which was contained in mangrove leaves. In another case, the highest cytotoxic activity was obtained by using the extract from *Nypa fruticans* extract plants, with an IC\(_{50}\) value at 180.186 \(\mu\)g / mL. In the selection of extracts and cell lines, further used as the follow-up tests, it should consider thoroughly the two main criteria: (1) that the extract must be able to inhibit cell proliferation without significantly affecting the direct extract’s cytotoxic effects and (2) the IC\(_{50}\) value of the extract must be lower than 200 \(\mu\)g/mL. Related to such, it turned out that the extract from *Nypa fruticans* plants could fulfill both criteria, and therefore was selected in the cytotoxic tests. As such in terms of cell proliferation, the WiDr cells which were used in the cytotoxic experiment only grew moderately, therefore regarded as a negative control.\(^{21}\)

Testing on the anti-cancer activity by *Centella asiatica* plant’s extracts (using water as extracting aqueous solvent) against the human colorectal cancer cell line (HCT116) has been carried out. As such, the cytotoxic evaluation was performed using the MTT test method. It turned out that the cytotoxic activity by the plant’s aqueous extract depended on the dose of HCT116 cells. Still related, the IC\(_{50}\) values achieved by the aqueous plant’s extract were 50 to 200 mg/mL, which indicated that the extract’s effect on colorectal cancer cells did efficaciously occur.\(^{22}\)

Research on natural renewable sources, among others so-called *Cuscuta reflexa* plant extract, revealed that the extract exhibited anti-proliferation activity on the HCT-116 cancer cells. Further, results of isolation on the *Cuscuta reflexa* plant extract disclosed that specific chemical compounds were identified, such as scoparone (1), \(p\)-coumaric acid (2), stigmasta-3,5-diene (3), and 1-\(O\)-\(p\)-hydroxycinnamoylgucose (4). Still related, the compounds 1-3 were strongly indicated to exert cytotoxic activity on the HCT-116 cancer cells with moderate intensity.\(^{23}\)

The concentration of the bioactive compounds (e.g., plant extract) as well as of the cells themselves is one of the parameters, which can significantly affect the viability of HCT116 cells. In another case, reduced exposure to emodin could affect cell viability. The IC\(_{50}\) value of the emodin was 47.50 ± 0.14 \(\mu\)mol /L.
According to the information about the IC\textsubscript{50} values, the apoptotic rate of HCT116 cells was checked after 48 hours of treatment with the emodin at 20, 40, and 80 µmol/L concentrations.\textsuperscript{24}

In relevant, Grape Seed Extract (GSE) cytotoxicity was evaluated by the Sulphorhodamine B (SRB) test and IC\textsubscript{50} value. The IC\textsubscript{50} value refers to a measure of the effectiveness of a compound (among others the plant extracts) in inhibiting the biological or biochemical functions of living organisms (e.g., cancer cells). Still related, human colon cancer HCT116 cells and normal human HOPE epithelial cells were treated with a serial dilution of GSE (23.43-3000 µg/mL). Results showed that after 72 hours of the treatment, the GSE could reduce the HCT116 cell proliferation (to the IC\textsubscript{50} value at 80µg/mL). However, unfortunately, the GSE (at low concentration) increased the growth of normal human WANT epithelial cells; and therefore, it was strongly presumed that GSE (at higher concentration, with the IC\textsubscript{50} value = 2000 µg/mL) could inhibit the epithelial cell proliferation.\textsuperscript{25} The same thing has also been strengthened by research that has been done on Italy and Palieri grape seed extract which has a higher anticancer effect than epigallocatechin and procyanidins.\textsuperscript{26}

The other research about the evaluation of the anticancer activity on the suspected cancer cells has been carried out by combining simple aromatic benzoate (SAB) and eugenol (EU) compounds against the colon cancer cells HCT-116. Further, the cytotoxic testing of SAB and EU on HCT116 and WiDr cells was performed using the MTT test method. Still related, the IC\textsubscript{50} values of a single compound called TFBA and EU consecutively in dealing with the proliferation of HCT116 cells were 0.35 and 22.3 µg/mL, respectively. Meanwhile, the IC\textsubscript{50} values of TFBA and EU against the WiDr cells were 0.29 and 26.7 µg/mL, respectively. Meanwhile, the EU - TFBA compounds combined brought out the lower IC\textsubscript{50} values (20.7 and 20.1 µg/mL) to deal with the proliferation of HCT116 and WiDr cells, respectively. These exciting research results show that a simple aromatic compound called TFBA afforded greater cytotoxic activity against the proliferation of the HCT116 and WiDr cell lines. Likewise, the EU - TFBA combination also showed greater cytotoxic activity on the HCT116 and WiDr cell lines than the other EU-SAB combinations. This research hence shows that TFBA compound, as well as EU-TFBA combined compound, exhibited significant potency as anticancer agents.\textsuperscript{27}

Another study research reported the cytotoxic activity of Curcuma longa plant extract against the HCT116 cancer cell lines using MTT assay. The results showed the IC\textsubscript{50} value at 78.46 µg/mL. These occurrences suggest that plant extracts afforded significant inhibition on HCT116 cell lines.\textsuperscript{28}

**CONCLUSION**

Phytochemical analysis on the extract of Saurauia vulcani plants revealed that it contained specific chemical compounds, among others tannins, saponins, flavonoids, and terpenoids. Chemical component analysis of ethyl acetate fraction showed the presence of fatty acid groups. Leaves’ ethyl acetate extract exhibited strong cytotoxic activities against the WiDr cancer cells (IC\textsubscript{50} value = 97.41 µg/mL), while the leaves’ methanol extract and the leaves’ n-hexane extract could also perform cytotoxic activities, but moderately to rather weakly (IC\textsubscript{50} values = 191.92 µg/mL and IC\textsubscript{50} value = 456.19 µg/mL, respectively). Conversely, the leaves’ methanol extract, the leaves’ ethyl acetate extract, and the leaves’ n-hexane extract so far exerted no cytotoxic activities against the HCT-116 cancer cells (IC\textsubscript{50} values = 529.39 µg/mL, IC\textsubscript{50} values = 568.53 µg/mL, and IC\textsubscript{50} value = 775.35 µg/mL, respectively). The best extract performance in the cytotoxic activities against the WiDr cancer cells was judged by the leaves’ extract using ethyl acetate fraction (IC\textsubscript{50} value = 97.41 µg/mL), followed by the methanol’s leaf extract as the second best (IC\textsubscript{50} value = 191.92 µg/mL), as both the IC\textsubscript{50} values were less than 200 µg/mL, which was regarded as one of the stipulated criteria. Accordingly, the ethyl acetate extracts and the methanol extracts from Saurauia vulcani leaves indicate their prospective potency and valuable efficacy as anti-cancer agents (cytotoxic activities) against the WiDr cancer cells.

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