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Exploring and evaluating the anti-cancer effects of *Tacca chantrieri*, the bat flower: A systematic review of experimental studies

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Abstract
Cancer is the second largest cause of death and the burden of cancer as well as cancer therapy on physical and mental health is ever increasing. The adverse effects of cancer chemotherapeutic agents are worsening the quality of life of cancer patients. Also, the availability of chemopreventive agents that have anti-cancer properties is scarce. Hence the research on such agents, derived from natural products with minimal side effects is invaluable. The present study is aimed at exploring the anti-cancer effects of *Tacca chintreiri* plant. We followed PRISMA guidelines to conduct the systematic review that included a total of 10 experimental studies. The present study indicated that *Taccalonolides, Evelynin, Taccachatrone, Chantriolides, Spirostanol saponins, Diarylheptanoids, Diarylheptanoid Glucosides* derived from the roots of *Tacca chintreiri* were found to have anti-cancer effects against human cancer cell lines. These compounds need to be further tested in clinical trials to evaluate and establish the anti-caner effects.

Keywords: *Tacca chinteriri*, bat flower, devil plant, cat whiskers, antiproliferative effects, anti-cancer effects, human cancer cell lines, phytomedicine

1. Introduction
India's projected cancer burden is expected to rise from 26.7 million DALYs (Disability adjusted life years) in 2021 to 29.8 million in 2025. Among non-communicable diseases, cancer contributes the most to the death rate (18.1%) next only to cardiovascular disease (63.3%) [1]. Cancer treatments that are local (surgery and radiation therapy) and systemic (chemotherapy, immunotherapy, hormone therapy) cause adverse reactions like neutropenia, lymphoedema, hair loss, nausea and vomiting, cancer pain, thrombosis, and cognitive ability problems [2]. Equally efficient and safe chemopreventive and chemotherapeutic agents from medicinal plants currently interest many physicians around the globe. The major challenge to the practical utility of such compounds is the lack of bioavailability. Various methods of extraction, storage, transport, and studies on synergy with other compounds supported the development of efficient treatment strategies for cancer. The plant-derived compounds currently used for cancer therapy are Vinca alkaloids, Podophyllotoxin derivatives, Taxanes, Campothecin derivatives, and Homoharringtonines. Some known plants that are proven to have anti-cancer benefits in vitro and in vivo were *Allium Sativum* (garlic), *Annona Muricata* (graviola), *Cannabis Sativa* (marijuana), *Camellia Sinensis* (Green Tea), *Gossypium Hirsutum* (cottonseed oil), *Hydrocotyle Asiatica (Gotukola), Hypericum Perforatum (St John's Wort), Mangifera Indica (mango), Oroxylum indicum (Sonapatha), Picrorrhiza kurroa (Kutki), Silybum Marianum, Zingiber Officinale* (ginger) etc [3]. The bat flower, scientifically called *Tacca chintrieri*, is one such potential plant with anti-cancer activity. *Tacca chantrieri*, belonging to the Dioscoreaceae family, was first described in 1901 by Édouard André. It is commonly known as the black bat flower due to its shape and color. Many in-vitro studies explored the different properties of bat flowers [4]. *Suchada Sudtiyanwimon et al*. proved that the saponin substances in the partial pure extract of T. *chintzier* possessed anti-inflammatory and anti-microbial effects [5]. Kittipong Keardrit *et al*. proved that the ethanolic extract of *T. chintreiri's* rhizome had analgesic, antipyretic, and anti-inflammatory activities [6]. The ethanols, saponins, and diarylheptanoid glucosides extracted from the plant roots were found to have hypotensive activity [7].

The plant extracts are being researched for anti-cancer activities. Few experimental studies were promising anti-cancer benefits of the plant. Evidence of the effectiveness of *T.chintreri* is scarce. To fill this research gap, the present study evaluates the anti-cancer benefits of *T.chintreiri*.

Materials and Methods

Search strategy: We systematically searched PubMed, Elsevier Science Direct, and Wiley Online Library databases from inception to June 2024 using MeSH terms "*Tacca chintreiri*" OR "Bat flower" AND "anti-cancer effects." We conducted the study following PRISMA guidelines. The review protocol was not registered prior.

Study selection

Eligibility criteria: Criteria for inclusion were (i) Original research articles published in the past 20 years. (ii) articles testing the anti-cancer effects of *Tacca chintreiri*. Criteria for exclusion were (i) articles available in languages other than English. (ii) Review articles. (iii) Articles that are available only as abstracts.

Quality assessment: All the authors methodologically and independently assessed the quality of the studies for adequate sample size, bias, and standardization. We used the operationalized Nature reporting checklist for bias assessment [8].

Data extraction: The extraction of data pertaining to authors, samples, techniques, results, and outcomes was done and synthesized in the form of narrative synthesis, which was later clustered and presented in a tabulated manner.

3. Results

Search results

A Systematic search following PRISMA guidelines in scientific databases using MeSH terms resulted in 21967 articles. 21278 were removed due to duplication, other languages, and reviews. 689 articles were checked for eligibility. The remaining 230 articles were screened for tiles and 73 for abstracts. After assessing quality, ten studies were included for qualitative synthesis [Fig: 1]. The characteristics of all included experimental studies were analyzed in terms of samples, experimentation techniques, results, and outcomes to provide a narrative synthesis. [Table 1]. Bias assessment indicated that all the studies had having low risk of bias [Table 2].

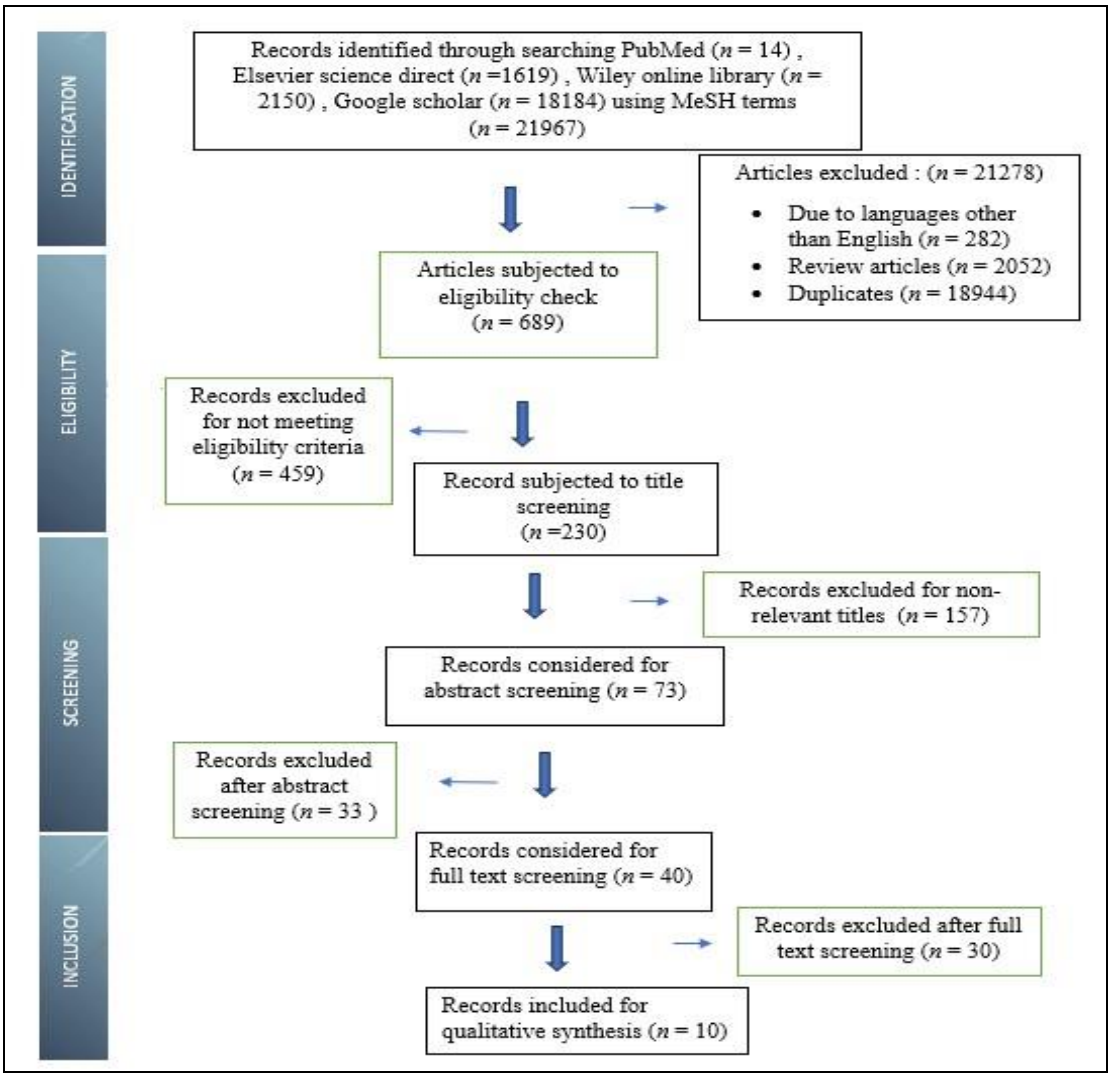

Fig 1: PRISMA flow chart showing the steps for inclusion of studies in the systematic review.

Table 1: Characteristics of included studies

Sl. No	Author, Year, Area	Design	Sample	Technique	Result	Outcome
1	Risinger A.L *et al* [9] 2013, San Antonio	In vitro study	MDA-MB-435 melanoma cancer cell line.	Bioassay-guided Chromatography fractionation of the taccalonolides. Their action on microtubules evaluated.	Taccabulin A directly inhibits tubulin polymerization by binding to the colchicine site on tubulin. It caused significant microtubule depolymerization and antiproliferative effects with an IC_{50} of 435 ± 14 nM.	Taccabulin A showed synergistic antiproliferative effects against melanoma cancer cells when combined with a Taccalonolide, which binds to a different site on tubulin.
2	Peng J *et al* [10] 2010, San Antonio	In vitro study	MDA-MB-435 melanoma,MDA-MB-231 breast, PC-3 prostate, and HeLa cervical carcinoma cells	A new benzoquinone-type *retro*-dihydrochalcone, named Evelynin, was isolated from the roots and rhizomes of *Tacca chantrieri*.	The IC_{50} value for synthetic Evelynin in human melanoma cell line was 4.1 (0.3 µM). Other cancer cell lines, including the breast cancer cell line, the prostate cancer cell line, and the cervical cancer cell line, showed similar sensitivities to evelynin, with IC_{50} values of 3.9 (0.1), 4.7 (0.4), and 6.3 (0.7) µM, respectively.	Evelynin exhibited cytotoxicity against melanoma, breast, prostate, and cervical carcinoma cells. Cytotoxicity of this compound was not due to cellular microtubules.
3	Ni G *et al* [11] 2015, Beijing	In vitro study	Human cancer cells of NCI-H1650-non-small cell lung, HepG2-hepatocelluar, BGC 823 stomach, HCT-116-colon, and A2780-ovarian carcinoma.	After HPLC-MC, EtOH extract from *T. chantrieri* was subjected to MCI gel (CHP 20P), silica gel, ODS, Sephadex LH20 column chromatography, and reversed-phase HPLC to extract six new Taccalonolides (1-6), two new Withanolides (7-8), and ten known compounds (9-18). The crude extract of was analyzed by HPLC-MS	Compounds 16, 8, 11, 12, 16, and 18 were inactive (> 10 µM). Compound 7 exhibited selective cytotoxicity against A2780 cell lines with an IC_{50} value of 4.61 µM. Compounds 9, 10, 13-15, and 17 exhibited cytotoxic activity, with IC_{50} values of 1.13-5.71 µM	The study found that compounds 9, 10, 14, 15, and17 which are Taccalonolides A, B, L, K, N, and R respectively, exhibited cytotoxicity against human lung , hepatocellular , stomach , colon, ovarian cancer cells.
4	Yena P. H *et al* [12] 2016, Vietnam	In vitro study	BV2 cells (substitute for primary microglial cells)	Two new steroidal glucosides, chantriolides D and E (1 and 2), along with four known compounds, chantriolide A (3), chantriolide B (4), chantriolide C (5), and (25S)-spirost-5-rhamnopyranosyl-glucopyranoside (6) were isolated from the rhizomes of *Tacca chantrieri*. Their structures were determined by 1D and 2D NMR spectroscopic and HR-ESI-MS data.	Compounds 1 and 2 showed a strong inhibitory NO effect in BV2 cells, with IC_{50} values of 12.45 and 59.03 µM, respectively. Compounds 3 - 6 did not show an inhibitory NO effect in BV2 cells, with an IC_{50}>100 µM	The study found that chantriolides D and E derived from rhizomes of *Tacca chantrieri* reduced systemic inflammation by decreasing the over expression of nitric oxide from microglial cells.
5	He J *et al* [13] 2021, Wuhan	In vitro study	A549 (lung cancer cells), SW480 (colorectal cancer cells), HL-60 (promyeloblast cells)and MCF-7(breast cancer cells).	Seven highly oxidized steroids, Taccachatrones A−G (1−7), together with four known Taccalonolides (8−11), were characterized from the rhizomes of *Tacca chantrieri*. The structures of 1−7 were established on the basis of spectroscopic data analysis, while the absolute configurations were determined by single-crystal X-ray diffraction.	Compounds 7, 8, 10, and 11 exhibited cytotoxicity to A549 with IC_{50} of 7.3 ± 0.03 , 2.8 ± 0.66 , 8.3 ± 0.29 ,7.5 ± 0.83 respectively, while compound 8 exhibited additional cytotoxicity to HL-60 and MCF-7 with IC_{50} of 1.6 ± 0.15 and 6.9 ± 0.12 respectively.	Compounds 7, 8, 10, 11 which are Taccachatrone G, Taccalonolides AI, T, and AW respectively, derived from rhizomes of *Tacca chantrieri* were cytotoxic against lung cancer cells. compound 8 was cytotoxic also to Promyeloblast and Breast cancer cells.
6	Yokosuka A *et al* [14] 2002 , Japan	In vitro study	HL-60 human promyelocytic leukemia cells, HSC-2 oral squamous carcinoma cells, and normal human gingival fibroblasts (HGF)	Two new diarylheptanoids (1, 2) and seven new diarylheptanoid glucosides (3-9) were isolated from the rhizomes of *Tacca chantrieri*. Their structures were determined by spectroscopic analysis. Cytotoxic activities were evaluated using a modified MTT assay method.	The diarylheptanoids 1, 2, and 7a, and the diarylheptanoid glucosides 3, 4, 6, and 7, with 3-4 phenolic hydroxyl groups, showed moderate cytotoxic activity against HL-60 cells with IC_{50} 1.8-6.4 µg/ mL. Those with 2 phenolic hydroxyl groups (5, 5a, 8, 8a, 9, 9a) did not exhibit apparent cytotoxic activity.	The compounds derived from *Tacca chantreiri* were found to be more cytotoxic against human oral squamous carcinoma cells when compared to normal human gingival fibroblasts. The cytotoxic activity was found to be more in compounds with 3 to 4 phenolic groups.
7	Yang Y *et*	In vitro	*Tert*-Butyl	11 highly oxidized Withanolides,	Compounds 5-	The compounds isolated

	al [15] 2022, China	study	hydroperoxide (*t*-BHP)-injured AML12 hepatocytes.	Chantriolides F-P (1-11) and six known analogues (12-17), were isolated from the rhizomes of *Tacca chantrieri*. Their structures were established by spectroscopic data analysis and their absolute configurations were confirmed by single crystal X-ray diffraction analysis.	11 and 16 significantly enhanced cell viability. Compound 8 decreased ROS and increased glutathione level in *t*-BHP injured AML12 hepatocytes by promoting nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2).	from roots of *Tacca chantrieri* were found to have hepatoprotective activity. They enhanced antioxidant activity by decreasing ROS and increasing Glutathione.
8	Peng J *et al* [16] 2011, San Antonio	In vitro study and In vivo study	HeLa cells (In-vitro) Syngeneic murine mammary carcinoma 16/C model (In-vivo)	3 new Taccalonolides, Z, AA, and AB, along with 2 known compounds, Taccalonolides R and T, were isolated from *Tacca chantrieri* and *Tacca integrifolia*. Taccalonolide structures were determined by 1D and 2D NMR methods. SRB assay was used to assess antiproliferative potential.	The most potent Taccalonolide is the newly identified Taccalonolide AA (IC_{50}=32.3 nM). Taccalonolides Z (120 nM), B (190 nM), N (247 nM), T (335 nM), A (594 nM) and E (644 nM). Taccalonolides AB (IC_{50}=2.8 µM) and R (IC_{50}=13.1 µM). were significantly less potent.	Microtubule stabilisation and mitotic arrest possessed by Taccalonolides AA, E, and N were found to have anti proliferative activity against cervical (in vitro) and mammary cancers (in vivo).
9	Yokosuka A *et al* [17] 2002, Japan	In vitro study	HL-60 human promyelocytic leukemia cells.	The rhizomes of *Tacca chantrieri* were analysed for steroidal saponins. 4 new *Spirostanol saponins* (1-4), and 1 known saponin (5) were isolated. Structures were elucidated by extensive spectroscopic analysis, nuclear magnetic resonance and hydrolytic cleavage.	Compounds 1 and 5 showed considerable cytotoxicity (IC_{50}=1.8 and 2.1 mM), whereas etoposide or positive control gave an IC_{50} of 0.37 mM. Compounds did not show cell growth inhibitory activity.	The saponin derivatives from the roots of *Tacca chantrieri* showed cytotoxic effects but not antiproliferative activity on human Leukemia cells.
10	Risinger A.L *et al* [18] 2009, San Antonio	In-vitro study and in -vivo RCT	In-vitro: HeLa, SK-OV-3(ovarian cancer) , MDA-MB-435 (breast cancer), NCI/ADR cell lines In vivo: 8-week-old female C3H mice (*n*=10)	Study utilized a supercritical fluid extractor with a solvent modifier followed by flash chromatography separations and HPLC purifications to isolate Taccas A and E. The Taccas A and E were each modified at the C15 position by mild base hydrolysis to generate Taccas B and N, respectively.	In-vitro study: Relative resistance values of 4.1, 12, 5.1 and 6.1, respectively were observed for Tacca A, B, E and N. In-vivo study: Tacca A had excellent antitumor activity when given at a total dose of 38 mg/kg, resulting in a 91% growth inhibition (9%T/C, 2.3 log kill and 9-day TGD)	These results demonstrate that Tacca A had excellent dose and timing-dependent antitumor potential against a highly drug resistant model in both in-vitro and in-vivo.

Table 1: Characteristics of included studies. HPLC-MS: high performance liquid chromatography-mass spectrometry; EtOH: ethyl alcohol. IC_{50}=maximal inhibitory concentration. NMR=nuclear magnetic resonance. HR-ESI-MS=High-resolution electrospray ionization mass spectrometry. ROS=reactive oxygen species. SRB=sulforhodamine B.

Table 2: Bias assessment by Nature reporting checklist.

	Risinger A.L *et al* [9]	**Peng J *et al* [10]**	**Ni G *et al* [11]**	**Yena P. H *et al* [12]**	**He J *et al* [13]**	**Yokosuka A *et al* [14]**	**Yang Y *et al* [15]**	**Peng J *et al* [16]**	**Yokosuka A *et al* [17]**	**Risinger A.L *et al* [18]**
Sample Selection & Experimental Setup	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Is the experimental unit clearly stated?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Replication	Yes	No	No	No	Yes	No	Yes	No	No	No
sex of the cells	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Source of cells	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Authentication of cell lines hela,hcc,ct-26, aml, hela	No	Yes	No	No	No	No	Yes	Yes	No	Yes
Contamination testing	No	No	No	No	No	No	No	No	No	No
Primary or continuous cell lines	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous
Passage of culture	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Blinding	No	No	No	No	No	No	No	No	No	No
Exclusion/attrition	No	No	No	No	No	No	No	No	No	No

Study results: Various compounds extracted from *Tacca chintreiri* exhibited anti-cancer effects on different types of human cancer cell lines [Table 3].

Table 3: Compounds of *Tacca chintreiri* with anti-cancer potential on various types of cancers

Cervical cancer cells	Evelynin & Taccalonolides A, N, E
Hepatocellular cancer	Taccalonolides A, B, L ,K, N, R & Withanolides, Chantriolides F-P
Breast cancer cells	Evelynin & Taccachatrone G, Taccalonolide AI, Taccalonolide T, Taccalonolide AW, Taccalonolides A, N, E
Colorectal cancer cells	Taccalonolide A , Taccalonolide B, Taccalonolide L , Taccalonolide K, taccalonolide N and Taccalonolide R & Taccachatrone G Taccalonolide AI, taccalonolide T, Taccalonolide AW.
Melanoma cells	Taccabulin A & Evelynin
Lung cancer cells	Taccalonolide A , Taccalonolide B, Taccalonolide L , Taccalonolide K, Taccalonolide N and Taccalonolide R & Taccachatrone G Taccalonolide AI, Taccalonolide T, Taccalonolide AW.
Brain cancer cells	Taccabulin A & chantriolides D and E.
Prostate cancer cells	Evelynin
Ovarian cancer cells	Taccalonolide A , Taccalonolide B, Taccalonolide L , Taccalonolide K, Taccalonolide N and Taccalonolide R & Tacca A
Leukemia cells	Taccachatrone G Taccalonolide AI, Taccalonolide T, Taccalonolide AW & *Spirostanol saponins* (1-4) and Saponin (5)
Oral cancer cells	Diarylheptanoids 1, 2 and Diarylheptanoid Glucosides 3-9

4. Discussion

Cells acquire certain biological capabilities white transforming into cancerous cells. These capabilities are known as cancer hallmarks. With their added therapeutic safety, natural plant products target these hallmarks to intercept the process of neoplastic transformation. The secondary metabolites derived from plants, also called Phytochemicals, have cancer-fighting potential through modulation of cancer signaling pathways, DNA repair mechanisms, and regulation of free radical production [19]. The current study evaluated such properties of *Tacca chintrieri*. The bioactive compounds were extracted in all experimental studies, their structures were studied, and Spectroscopic analyses, HPLC-MS, and X-ray diffraction analyses confirmed the configuration. MTT assays, NMR, and SRB analyses measured their cytotoxic activity. Compounds derived from the roots of *T. chintreiri* were found to have anticancer effects. *T. chintreiri*, also called bat flower, cat whiskers, and devil flower is rarely available and native to Southeast Asia. Current research supports its anticancer, antifungal, Analgesic, Antipyretic, and Anti-Inflammatory actions [20]. According to a chemical analysis by Akihito Yokosuka *et al*, rhizomes of *T. chintreiri* produced secondary metabolites, namely, diarylheptanoids, diarylheptanoid glucosides, steroidal glycosides with the aglycone structures of Ergostane, Withanolide, Spirostan, Furostan, Pseudofurostan, and Pregnane, as well as a Phenolic glucoside. Some Diarylheptanoids and Steroidal glycosides showed cytotoxicity against human cancer cells. These compounds may be possible leads for new anticancer drugs [21]. A systematic review was conducted by Christian Bailly *et al* to evaluate the anticancer potential of *Tacca plantaginea*. Steroids such as Taccanolides, which interfere with microtubules and sapogenins, contain potent anticancer activities [22]. Another study by Jedsada Maliwong *et al*. explained the extraction of cytotoxic saponins from rhizomes of *T. integrifolia*. The compounds of *T. chintreiri* had similar anticancer potential to chemotherapeutic drugs Taxanes. Taccalonolides derived from bat flowers can overcome resistance developed due to Taxanes and cross the blood-brain barrier [23]. Among the derived compounds, Taccalonolides had poor bioavailability due to their low water solubility and high metabolism whereas the glycosides, such as Taccaside and Taccaloside, had moderate bioavailability, with peak plasma concentrations within 1-2 hours after oral administration. New approaches like Nanoparticle and Liposomal formulations and prodrug approaches are being explored to improve the bioavailability. Natural compounds with microtubule-stabilizing abilities, like vinblastine, vincristine, and colchicine, are currently used in cancer chemotherapy despite the adverse effects. The pentacyclic steroids taccalonolides isolated from *T. plantaginea* (Hance) Drenth, *T. chantrieri* Andre, and *T. paxiana* can be developed to serve as alternative agents with minimal adverse effects [24, 25, 26]. The current study findings also suggested the low IC_{50} values of the derived components. All the ten studies included were experimental in vitro studies. Evidence supporting the anticancer effects of *T. chintreiri* obtained from the present study would provide scope for further research on compounds derived from roots that particularly exhibited anticancer properties in vivo studies in animals and humans. Proper sourcing, extraction methods, storage, and delivery of the compounds to achieve improved bioavailability and therapeutic efficiency need to be the directive for further research on *T. chantreiri*. The current was limited by the small number of studies included.

5. Conclusion

In conclusion, compounds derived from the roots of *Tacca chintreiri*, especially *Taccalonolides*, *Evelynin*, *Taccachatrone*, *Chantriolides*, *Spirostanol saponins*, *Diarylheptanoids*, *Diarylheptanoid Glucosides* were found to be effective anti-cancerous agents in vitro against human cancer cells.

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