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Review Article **Zoology**

Bioactive Compounds of Tinospora Cordifolia: Implications for Cancer Treatment and Disease Management

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Abstract

Tinospora cordifolia, or "guduchi," is a reported medicinal plant of high therapeutic value. Historically, it was utilized in Ayurveda and other herbal medicinal therapies, where it is valued for its efficacy in treating a range of diseases, such as metabolic disorders, infectious diseases, autoimmune diseases, and cancer. This manuscript presents the pharmacological significance of T. cordifolia, where its multi-dimensional bioactive molecules, such as alkaloids, diterpenoid lactones, and steroids, are responsible for its therapeutic actions. Recent scientific studies show its chemopreventive value, especially in regulating cellular proliferation and apoptosis. With the growing interest in the world for plant-based therapeutics, more studies are needed to prove its efficacy, to explain its mechanisms of action, and to determine its potential in today's medicine.

Keywords: Tinospora cordifolia (TC), bioactive components, alkaloids, diterpenoid lactones, steroids, cancer, heat shock proteins, molecular chaperones, chemopreventive agents, BMC proliferation, colony formation, C6 glioma cells, Benzo(a)pyrene (B(a)P).

INTRODUCTION

It was reported by WHO that the total 80% of the world population depends mainly on conventional medicines, in which the extracts of plants or their active constituents are used. From the beginning of human civilization, plants, which primarily relate to treatment, have been extensively used by humans for their therapeutic value. There has always been a lot of interest in discovering the active ingredient in plant materials to replace imitation pharmaceuticals and reduce their deleterious impacts. Nature has been employed as a source of therapeutic agents for thousands of years, and many contemporary medications have their origins in natural substances. In communities with limited resources, traditional medicine has continued to be the most efficient and practical form of therapy [1]. Following research on herbal remedies, it has sparked a variety of endeavours over the past few decades, with a focus on finding novel candidates to treat diseases that are already life-threatening. Medicinal and aromatic plants, which have economic importance, have a vital role in easing human suffering. Both in advanced and developing nations, the need for medicinal herbs has been rising daily. Among that, Tinospora cordifolia has a diverse array of bioactive components, and it has been proved an important medicinal plant, which has not yet received reasonable scientific attention [2]. The naturally derived shrub plant T. cordifolia, a member of the moonseed family Menispermaceae, is far more widely popular as "guduchi.". This plant has a great worth in terms of chemical constituents present in it and in Pharmacology. Tinospora cardiflolia is a substantial deciduous climbing shrub that can be found all over India, as well as in Sri Lanka, Bangladesh, and China [3].

The phenomenal ayurvedic herb T. cordifolia is used to treat a wide range of illnesses, including gonorrhoea, diabetes, leprosy, anaemia, rheumatoid arthritis, dermatological conditions, secondary syphilis, cancer, gout, jaundice, asthma, as well as bone fractures, liver & intestinal disorders, purifies the blood, and revitalises the entire body [4].

Phytochemistry:

The chemicals derived from T. cardifolia associate to various classes such as steroids, glycosides, alkaloids, polysaccharides, diterpenoid lactones, and aliphatic compounds as shown in fig 1. The compounds that have been isolated from this plant include arabinogalactan polysaccharide, palmarin, cordifolisides A to E, X gilosterol, picrotene, tinosporone, octacosonal, tinosporic acid, cordifol, chasmanthin, heptacosanol, palmatosides C and F, tinosporide, and columbin [3]. Alkaloids, such as choline, magnoflorine, isocolumbin, palmetine, tinosporin, jatrorrhizine, aporphine alkaloids, tetrahydropalmatine, and berberine, are present in the constituents isolated from the stem and root of T. cordifolia and have been shown to have anti-diabetes, anti-cancer, anti-viral, anti-psychiatric and anti-inflammatory. Diterpenoid Lactones,furanolactone, Cleodrane derivatives [(5R, 10R)-4R-8R-dihydroxy-cleroda-13(16), 14-dieno-17, 12S:18, 1S-dilactone], columbin tinosporides, jateorine and tinosporin, are also found in T. cordifolia. They exhibit biological properties that include anti-viral, anti-inflammatory, vasorelaxant, anti-hypertensive, and anti-microbial. Steroids (δ -sitosterol, Makisterone A, B-sitosterol, Ecdysterone, 20 -hydroxyecdysone) are found in the shoots of T. cordifolia. In early inflammatory arthritis, they all show effective responses in glucocorticoid-induced osteoporosis. Cell cycle arrests at the G2/M phase due to these components, and c-Myc reduction delays TNF-, IL-1, IL-6, and COX-2 production, as well as apoptosis [5].

Medicinal Properties of Tinospora cordifolia:

Several active compounds have been discovered in various parts of the T. cordifolia plant. These chemicals exhibit anti-cancer, anti-inflammatory, antifungal, anti-aging, antibacterial and antioxidative measures in fighting against disease as illustrated in fig. 2.

Cancer:

Being cancer a complex disease, there are various mapping changes involved in cell physiology, which eventually lead the way toward malignant tumors. The incursion of surrounding tissues and far off organs by tumor cells is the foremost cause of morbidity and mortality for most cancer patients [6]. The retardation in cancer treatment progress upraises great concerns, especially for notable investment which made into research [7].

Cancer can be categorized as an important hurdle in almost the whole countries of world for increasing the life expectancy. WHO estimates in 2019, that cancer is now become the first or second leading reason for death, almost in 112 of 183 countries and in further 23 countries, it rank third or fourth. Colon, breast, lung, rectum, and prostate cancer are the most prevalent forms of cancer [8]. Nearly about 10 million deaths occured in 2020 as cancer is a top most cause of death worldwide. From recent surveys, it confirmed that round about 400 000 children diagnosed with cancer each year. The World Health Organization (WHO) reported that there were over 18.1 million new cases of cancer in 2018, and 9.6 million people died from the disease. [7] Pakistan is present in the topmost in Asia for breast cancer, and this rising trend suggests that it is likely to increase, but if we take steps forward to remove the hurdles for early screening. Breast cancer was diagnosed in almost 26,000 women and about 13,500 women died in 2020, according to the World Health Organization [9].

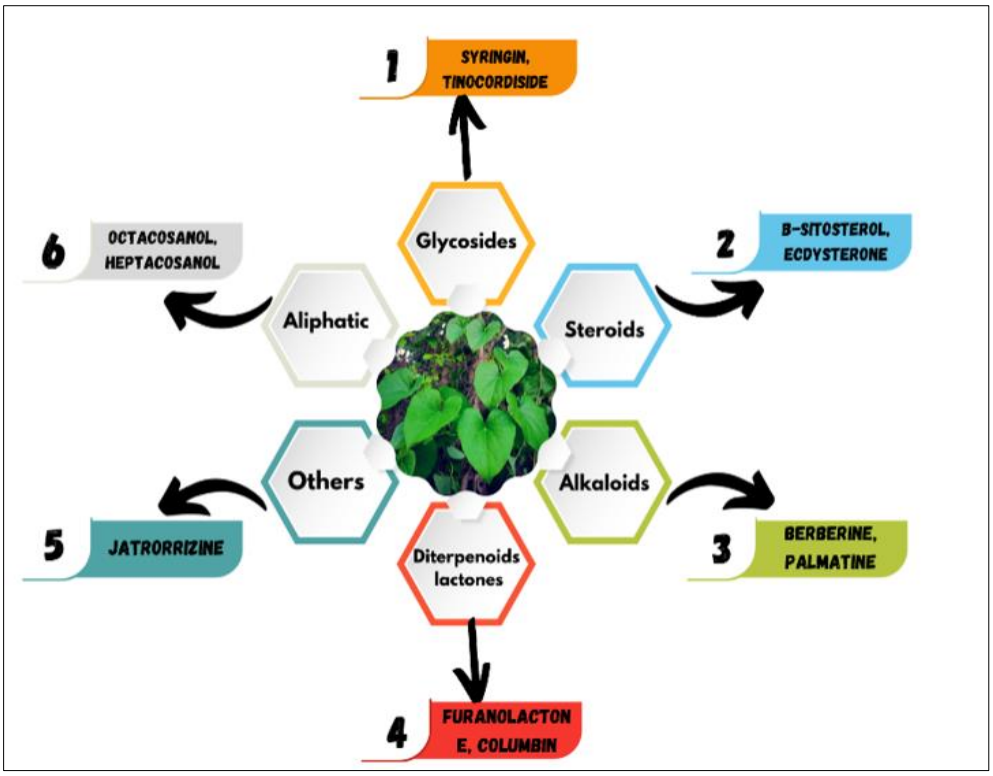

Fig. 1: Phytochemistry of Tinospora Cardifolia Plant

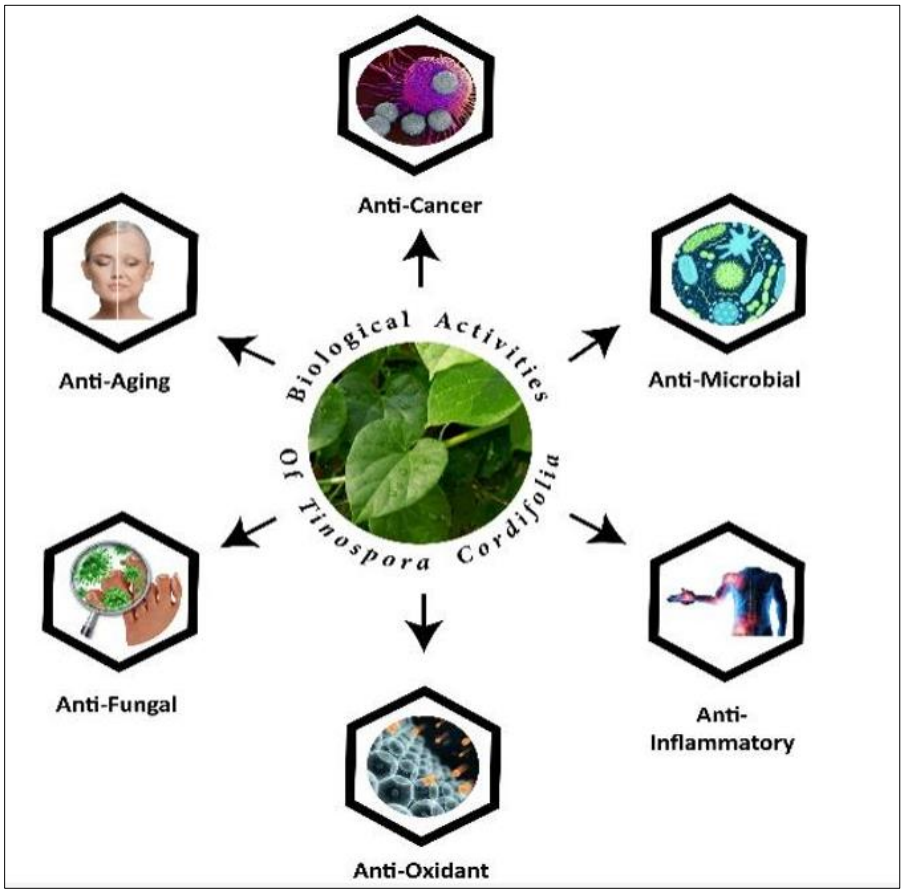

Fig. 2: Medicinal properties of T. Cardifolia

Various studies have centered their attention towards the anti-cancer activities of the compounds that occur naturally. Therefore, Plants that are regarded as a vital origin of active compounds are used in the

conservative medicine system, and play an important role in chemotherapy. The growing method to cure, counter or retard cancer is cancer chemoprevention with naturally occurring phytochemical extract. Hence, in search of effective chemopreventive agents, zero attempts are being performed to enhance the effectiveness of cancer treatments [10].

Anticancer activity:

Still regarded as the most horrible disease of the 20th century, cancer is becoming a more serious concern in the 21st. With one in four people having a lifetime risk of acquiring cancer, its prevalence has alarmingly reached new heights [11]. This disease is a major worldwide health issue affecting industrialized and developing countries. Many anti-cancer medications, including vincristine and etoposide, are used to treat cancer. Promising anti-cancer drugs have also been developed, including silvestrol, betulinic acid, combretastatin A-4, roscovitine, and flavopiridol [12]. Numerous herbs, including Tinospora cordifolia (T. cordifolia), have been studied for their potential to prevent cancer. The anti-cancer efficacy of T. cordifolia has been extensively studied in both in vitro and in vivo contexts. Numerous investigations have shown that T. cordifolia extracts and chemical compounds have anti-proliferative properties. Moreover, they have demonstrated effectiveness in animal cancer models [13].

Palmieri et al., (2019) showed that the extract of dried powder stem of T. cordifolia with solvent 2L of MeOH/H2O (1:1) can prevent the expression of numerous genes related to colon cancer's onset and spread. The levels of 44 selected genes in the human colon adenocarcinoma (HCA-7) cell line were measured by using real-time polymerase chain reaction (PCR). Thirty-three distinct genes involved in cell cycle and differentiation in vitro were downregulated (shown in fig 3) due to the BBR treatment in a time- and dose-dependent manner. Observing results suggested that the presence of BBR in the expression of various genes linked to the formation and growth of colon cancer was suppressed by T. cordifolia extract [14]. *Patil et al., (2021)* evaluated the effects of T.cordifolia extract on oral cancer cell lines. AW13516 cells, an oral cancer cell line, treated for 24 hours in vivo with aqueous extract of T. cardifolia at doses ranging from 5 lg/ml to 100 lg/ml and compared to a control (cells without treatment). T. cardifolia aqueous extract was observed to trigger apoptosis in AW13516 cells in a concentration-dependent manner, even at a very low concentration of 5 lg/ml. The treatment of the cells with the extract of T.cardifolia for 24 h revealed a significant decrease in the expression of epithelial-mesenchymal transition genes in a dose-dependent manner [15]. Mohan, V., & Koul, A. *et al.,* (2021) evaluate the chemopreventive efficacy of T cordifolia stem aqueous extract and its active components; Arabinogalactan (AG) acts against Benzo(a)pyrene [B(a)P], which triggered lung carcinogenesis in BALB/c mice (in vivo). B(a)P results in notable modification in carcinogen metabolizing enzymes (CMEs), which lead to reducing the glutathione and the lipid peroxidation levels as illustrated in Table 1.1. However, Aq. T. cardifolia and Arabinogalactan consequentially assist in restoring the altered levels of carcinogen metabolising enzymes and antioxidant machinery. Benzo (a) pyrene + aquatic extract of gudichi and Benzo (a) pyrene + Arabinogalactan treated groups unveil the classical aspects of apoptosis. Aquatic extracts of T cordifolia and Arabinogalactan (AG) are to be used as essential chemopreventive agents [10]. The production of silver nanoparticles (AgNPs) using plant extracts provides a strategy that is natural, affordable, and easily accessible. During the synthesis process, the phyto-constituents found in these plants serve as capping, stabilizing, and reducing agents. In this study, Tinospora cordifolia's aqueous leaf extract was used to investigate the production of AgNPs. Numerous methods, including Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy (TEM), energy-dispersive X-ray analysis, and X-ray diffraction, were used to characterize the produced AgNPs.

The spherical morphology of the produced AgNPs, which ranged in size from 25 to 50 nm, was validated by TEM investigation. The anticancer potential of these AgNPs, which were produced using T. cordifolia leaves, was then assessed by the study against the human lung adenocarcinoma cell line A549. The evaluation included several assays, such as the MTT and trypan blue assays, as well as the use of Annexin V-FITC and Propidium iodide (PI) to observe apoptotic morphological changes and DAPI (4,6-diamidino-2-phenylindole dihydrochloride) staining to examine nuclear morphological changes, measure reactive oxygen species generation, and determine mitochondrial membrane potential.

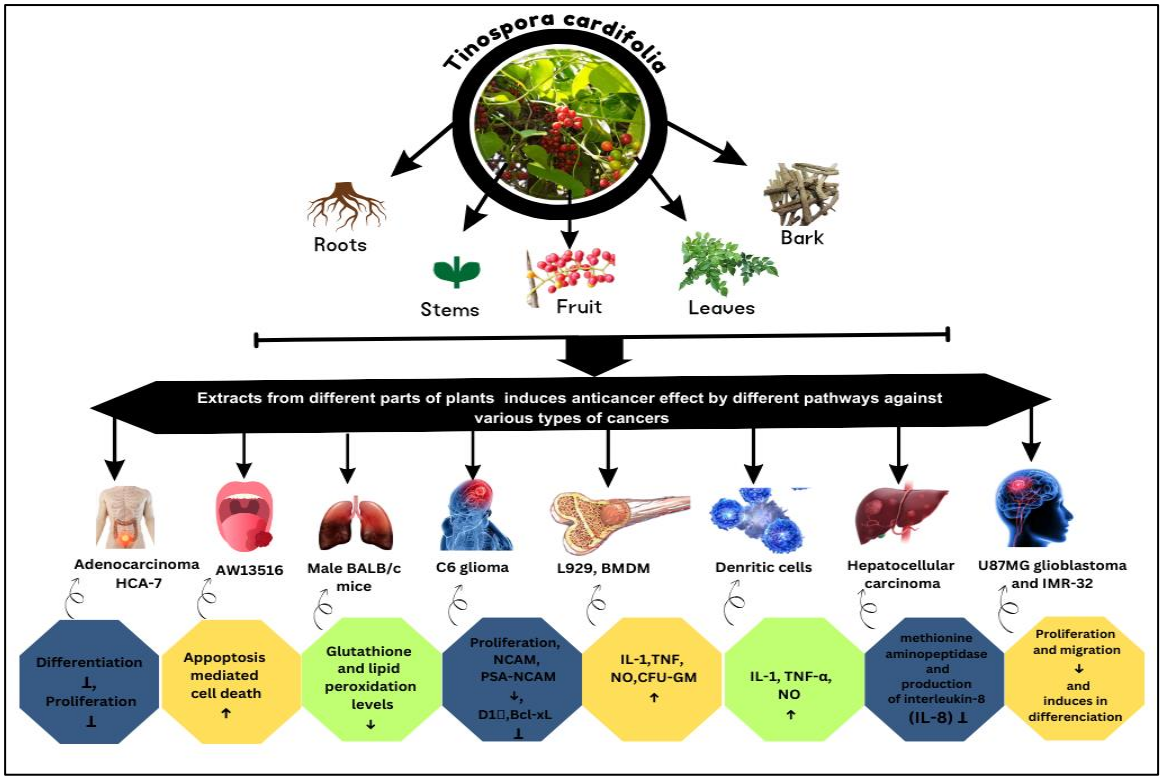

Fig. 3: Anticancer activities of different parts of T. cardifolia

These outcomes emphasize the potential use of AgNPs made from T. cordifolia leaves in the treatment for cancer by indicating that it is a highly toxic agent against lung adenocarcinoma [16].

Ansari et al. (2017) evaluated three cell lines: MDA-MB-231, MCF-7 (both breast cancer cell lines), and HACAT (a normal skin cell line) in order to determine the anti-proliferative effect of the chloroform fraction of Tinospora cordifolia (TcCF). To find out how different TcCF concentrations affected the proliferation of these cancer cells, they looked into a variety of concentrations, from 6.25 µg/mL to 100 µg/ml. The outcomes demonstrated that, at all tested concentration ranges, TcCF reduced the development of cancer cells. The levels of two proteins linked to cell survival and proliferation, Bcl-2 and c-myc, decreased following a 24-hour treatment with TcCF as expressed in Table 1.1. On the other hand, there was an increase in the amounts of p21, p53, Bax, and Bad, which are connected to apoptosis and cell cycle arrest. Moreover, the investigation revealed that TcCF therapy increased intracellular reactive oxygen species (ROS). This suggests that oxidative stress is induced in cancer cells by TcCF.

Overall, the in vitro research demonstrated TcCF's pro-apoptotic and antiproliferative effects on cancer cells. These results indicate TcCF's potential for use as a therapeutic agent in cancer management. Additional research is necessary to clarify the underlying mechanisms and assess TcCF's effectiveness in preclinical and clinical contexts [17].

In the present study, Ahmad *et al.*, (2015) employed a 50% methanolic extract of Tinospora cordifolia stem to characterize its in vitro cytotoxic effects against two cell lines: a normal Vero epithelial cell line and the human breast cancer cell line MDA-MB-231. T. cordifolia demonstrated cytotoxic and anticancer properties against the human breast cancer cell line at a 50 g/ml concentration. MDA-MB-241. [18] *Rashmi et al., 2019* used the TNBC cell line (MDA-MB-231) to test the effectiveness of Bis (2-ethyl hexyl) 1H-pyrrole-3,4-dicarboxylate (TCCP), a chemical found in T. cordifolia leaves. Internal ROS production, calcium elevation, and p53 phosphorylation were all results of TCCP therapy. The proteins (Bcl-2 and Bax) are found downstream and also in MPTP, cardiolipin peroxidation, and mitochondrial membrane depolarization. An increase in the sub-G1 population, apoptosis induction, and morphological changes were also noted. Additionally, TCCP in vivo decreased tumour burden and enhanced survival in mice with Ehrlich ascites tumour (EAT) by as much as a factor of two. After p53 was fixed, TCCP stopped the growth of tumours in EAT by killing MDA-MB-231 cells in vivo through reactive oxygen species (ROS) and mitochondrial-mediated apoptosis [19].

Tumor:

A tumor denotes an anomalous proliferation of tissue characterized by cells exhibiting accelerated growth compared to their normal counterparts, devoid of any inherent physiological function [20]. Many people experience headaches as a symptom. It is essential to understand that not all headaches are a sign of a brain tumor. However, it has been noticed that individuals

diagnosed with brain tumors frequently mention having headaches [21]. While brain tumors are not common, the presence of a brain tumor can often be associated with the occurrence of headaches.

Anti tumor effects:

In relation to brain tumors and other types of tumors, Tinospora cordifolia compounds and extracts have shown significant effects. Research studies have explored the effectiveness of T. cordifolia in the treatment of various tumor types, including brain tumors. These investigations have revealed promising outcomes, suggesting the effect of T. cordifolia as a therapeutic intervention for tumors.

Additional research is imperative to comprehensively grasp the mechanisms of action involved and assess the effectiveness of Tinospora cordifolia compounds and extracts in addressing brain tumors and other varieties of tumors. This research may contribute to the development of novel treatment approaches and enhance our understanding of the therapeutic potential of T. cordifolia in cancer management. In a study conducted by *Mishra et al. in 2013*, the anti-brain cancer activity of Tinospora cordifolia evaluated using C6 glioma cells. The researchers aimed to assess the potential of TCE on cell proliferation, migration, invasion, cell cycle, and apoptosis. The study findings demonstrated that TCE (Tinospora cordifolia extract) effectively impeded the proliferation of C6 glioma cells in a dose-dependent manner over a 72-hour duration. TCE effectively suppressed cell growth at 250 and 350 µg/mL concentrations. Furthermore, the expression of Mortalin, a stress indicator, increased after TCE treatment. The wound scratch experiment revealed that TCE treatment decreased the expression of NCAM, PSA-NCAM, MMP-2, and MMP-9, indicating its anti-migratory and anti-invasive capabilities. Cell cycle analysis demonstrated that TCE caused cell cycle arrest in C6 cells, specifically in the G0/G1 and G2/M phases. Additionally, the repression of cyclin D1 and Bcl-xL, which are associated with cell cycle progression and anti-apoptotic processes, contributed to the induction of apoptosis in the C6 cells. These findings indicate that TCE possesses significant anti-brain cancer activity against C6 glioma cells. The capacity of TCE (Tinospora cordifolia extract) to impede cell proliferation, migration, and invasion, coupled with its ability to induce cell cycle arrest and apoptosis, underscores its promising potential as a therapeutic remedy for brain cancer. However, further research is necessary to elucidate the underlying molecular mechanisms as shown in fig 3 and to assess the potency and safety of TCE in preclinical and clinical settings [22].

In a 2006 study, Singh *et al.,* investigated the in vivo effects of an alcoholic extract of the whole plant of Tinospora cordifolia (ALTC) using bone marrow cells (BMC) from mice with Dalton's lymphoma (DL). The researchers aimed to assess the impact of ALTC on cell proliferation and differentiation.

The findings indicated that when ALTC was used in lab tests at a dose of 100 mg/kg, it significantly boosted the growth and ability to form colonies of bone marrow cells (BMC) when exposed to L929, a substance that stimulates colony formation. This effect was observed in both mice and mice, with DL bearing tumors. These findings suggest that ALTC has the potential to enhance BMC growth and colony formation. Moreover, when mice received ALTC of phosphate buffered saline (PBS), there was an increase in the number of granulocyte macrophage colony stimulating factor (GM-CSF) in their bone marrow cells (shown in Fig. 3). Additionally, mice injected with ALTC along with exposure to light at night. When the temperature fluctuates, macrophages produce interleukin 1 (IL 1) and tumor necrosis factor (TNF). These macrophages are derived from the bone marrow stimulated by lipopolysaccharide (LPS). These results demonstrate that ALTC can stimulate BMC growth, colony formation and cytokine production by bone marrow derived macrophages. These findings suggest that ALTC may possess properties. Further research is imperative to understand the underlying mechanisms and explore the applications of ALTC in lymphoma treatment and immune regulation [23]. *Singh et al*., conducted a study in 2005 to examine how tumor-associated macrophages (TAM) react to granulocyte macrophage colony stimulating factor (GM-CSF), interleukin 4 (IL-4) and tumor necrosis factor alpha (TNF α) in a laboratory setting. The objective was to assess whether these factors could induce the differentiation of TAM into dendritic cells (DC). The researchers also examined the impact of an alcoholic Tinospora cordifolia (ALTC) extract on TAM differentiation into DCs. The study findings indicated that TAMs have the ability to transform into DCs when exposed to GM-CSF, IL-4 4 and TNF-α, in a laboratory environment. It is interesting to note that the ALTC extract was discovered to promote the differentiation of TAMs into DCs by releasing GM CSF, IL 4 and TNF α. This suggests that ALTC has the potential to modulate the differentiation of TAM into DCs, which are crucial for immune responses.

In vivo experiments involving the administration of Tinospora cordifolia extract were conducted on living organisms. Two days following tumour transplantation, ALTC injection (200 mg/kg body weight) increased tumour cytotoxicity and vitro produced TNF-α, IL-1, and nitric oxide (NO) in DCs derived from TAM, illustrated in figure 3. Furthermore, administering these differentiated DCs to mice bearing Dalton's lymphoma increased the likelihood of the mice surviving the tumor. These findings highlight the immunomodulatory potential of Tinospora cordifolia extract on TAM differentiation into DCs and its potential implications in enhancing antitumor immune responses. Further research is necessary to elucidate the underlying

mechanisms and to explore the therapeutic potential of Tinospora cordifolia in cancer immunotherapy [24].

Angiogenesis:

Angiogenesis, the complex development of new capillary blood vessels, which is crucial to many physiological and pathological states. Importantly, the process of tumor-induced capillary formation is strikingly similar to capillary proliferation in non-malignant angiogenesis [25]. Angiogenesis begins in an adult organism with a well-coordinated cascade of events that includes EC activation, vascular basement membrane degradation, and interstitial matrix vascular development [26].

Rashmi, K. C. and Atreya conducted studies that provided evidence of the significance of heat shock proteins (HSPs) and molecular chaperones in cancer cells. These proteins hold pivotal importance in facilitating the appropriate functionality of diverse oncoproteins engaged in cell proliferation, migration, tumor angiogenesis, and cellular survival. The anti-angiogenic effect of a compound called pyrrol (TCCP) derived from T. cordifolia was investigated both ex vivo and in vivo. The ex vivo study utilized the rat corneal micro-pocket assay, while the in vivo study was conducted in tumor-bearing mice. The results demonstrated that T. cordifolia compound pyrrol exerted an anti-angiogenic effect. In several types of cancers, HSPs are overexpressed due to the activation of transcription factors, particularly Heat-shock factor 1 (HSF-1). The downregulation of HSP90 by T. cordifolia compound pyrrol was observed, which led to the immobilization of HSF-1. Consequently, this caused the retardation of VEGF-induced cell migration, a decline in tumor cell proliferation, and a subsequent decrease in tumor burden and neo-angiogenesis. These findings suggest that the compound derived from T. cordifolia has potential in modulating HSP90 activity and inhibiting angiogenesis in tumors [27]. Nagaraj *et al., (2012)* explored the pivotal connection of angiogenic growth factors like Vascular Endothelial Growth Factor (VEGF) and Metastasis Associated Protein 1 (MTA1) in governing cell migration, invasion, and proliferation—fundamental processes in metastasis. Studies have specifically delved into examining the anti-angiogenic properties of the hexane fraction sourced from T. cordifolia stems. To validate the anti-angiogenic efficacy of the hexane fraction, experiments were conducted using Human Umbilical Vein Endothelial Cells (HUVECs) for tube formation and the rat cornea angiogenesis model. The results confirmed that the hexane fraction of T. cordifolia stems exhibited anti-angiogenic activity. Further investigations focused on the effects of the hexane fraction and SB202190, a MAP kinase blocker, on the metastatic processes facilitated by VEGF or MTA1. The studies conducted on MDA-MB-231 cells, which are known for their proliferation, invasion, and migration abilities, demonstrated that both the T. cordifolia hexane fraction and SB202190 significantly inhibited these metastatic processes. These findings suggest that the hexane fraction derived from T. cordifolia stems possesses anti-angiogenic properties and can effectively impede the cell proliferation, invasion, and migration associated with metastasis, particularly in the presence of VEGF or MTA1 [28].

Carcinoma:

Presently, hepatocellular carcinoma (HCC) is the leading cause of death among cirrhotic patients and a major factor in cancer-related mortality generally. The results show that metabolic syndrome in conjunction with non-alcoholic liver disease can be a significant cause of hepatocellular carcinoma, including alcohol-induced liver damage and viral hepatitis. [29] Along with other known risk factors including alcohol intake and viral infections, metabolic syndrome and non-alcoholic liver disease should be considered as potential risk factors for the development of hepatocellular carcinoma (HCC). Better methods of preventing and managing hepatocellular carcinoma can be developed by acquiring a deeper knowledge of these connections.

Dhanasekaran and Baskar et al., (2009) undertook a study involving the isolation of a diterpenoid compound named (5R, 10R)-4R, 8R-dihydroxy-2S, 3R:15, 16-diepoxycleroda-13(16), 17, 12S:18, 1S-dilactone (ECD) from the stem of Tinospora cordifolia utilizing alcohol-based extraction methods. They then investigated the chemopreventive potential of ECD in an in vivo model of hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DEN). Fifty male Wistar rats were segregated into five distinct groups for the investigation. Group I functioned as the normal control. Groups II to IV were subjected to DEN (0.01% in drinking water) over a span of twenty weeks to induce HCC. Group III received ECD (10 mg/kg body weight) as a preventive measure throughout the entire duration of the study. Group IV received ECD (10 mg/kg body weight) solely for the final eight weeks of the study. Group V was exclusively administered ECD alone (10 mg/kg body weight) for the entire duration of the experiment. Following the study's completion, all Wistar rats were euthanized, and their biochemical endpoints were scrutinized to evaluate the impact of ECD treatment on rats with DEN-induced hepatocellular carcinoma (HCC). The outcomes unveiled that ECD treatment elevated antioxidant and detoxifying enzyme levels, concurrently diminishing serum transaminase and hepatic marker enzyme levels, thereby restoring them toward normalcy in both the preventive and curative cohorts. Histopathological scrutiny and nodular incidence examination corroborated that ECD markedly reduced tumor occurrence. These findings suggest that ECD derived from T. cordifolia exhibits chemopreventive properties against hepatocellular carcinoma. ECD treatment enhanced antioxidant defenses, improved liver function, and effectively reduced tumor formation in DEN-induced HCC rats [30].

Proliferation:

"Proliferation" denotes the phenomenon of tissue cell growth and stands as a pivotal process in various diseases. In the case of cancer, the abnormal proliferation of cancer cells occurs through their increased rates of cell division and multiplication [31]. Understanding the dynamics of cellular proliferation is essential for effective cancer management, as it is an important aspect of the cancer phenotype. The introduction of in vitro assays to measure tumour growth rates has provided researchers with useful instruments for examining and evaluating tumour progression [32]. We can learn more about the processes that drive cancer cell proliferation and how to develop more effective targeted treatments with the use of these tests. Polu, P. R., and Nayanbhirama *et al.,* (2021) embarked on a study to investigate the prospective properties of diverse fractions extracted from the stems of Tinospora cordifolia (T. cordifolia). Ethanol extract (TCE), petroleum ether (TCP), dichloromethane (TCD), n-butanol (TCB), and aqueous (TCA) fractions were meticulously prepared and subjected to assessment regarding their flavonoid and phenolic content, alongside an evaluation of their antioxidant activity utilizing varied methodologies. The study assessed fractions from Tinospora cordifolia on HeLa cervical carcinoma cells, finding n-butanol fractions and ethanol extract demonstrated superior antioxidant activity in various assays. With IC50 values of 14.81 ± 0.53, 29.48 ± 2.23, 58.20 ± 0.70, and 21.17 ± 1.19 for diphenylpicryl, ABTS, nitric oxide, and iron chelating activities, respectively, n-butanol fractions were particularly effective. Furthermore, the MTT assay (IC50 = 54.23 ± 0.94 µg/mL and 101.26 ± 1.42 µg/mL, respectively) and SRB assay (IC50 = 48.91 ± 0.33 µg/mL and 87.93 ± 0.85 µg/mL, respectively) demonstrated the significant cytotoxicity of TCD and ethanol extract from T. cordifolia against HeLa cells. The study highlighted the dichloromethane fraction as the most potent in inhibiting proliferation. Ongoing investigations aim to unveil underlying mechanisms and isolate active components accountable for these effects. The results show that the n-butanol and dichloromethane parts of T. cordifolia may have antioxidant and anti-proliferative effects. The specific mechanisms at activity and the chemicals that are accountable for these effects will hopefully be better understood in future research [33].

As a model system (in vitro), Sharma, A., Saggu, S. K., Mishra tested T. cordifolia stem extracts in U87MG glioblastoma and IMR-32 neuroblastoma cells to see if either induced differentiation. For GFAP and MAP-2, MTT assays indicated a valuable decrease in cell proliferation and differentiation induction from Chloroform (Chl-TCE) and hexane (Hex-TCE) extracts in glioblastoma and neuroblastoma, accordingly. Moreover, the expression of stress indicators HSP70 and Mortalin was upregulated and senescence was triggered by both extracts. Chloroform and hexane extracts impede the migration of both the U87MG glioblastoma and the IMR-32 neuroblastoma, as shown by the wound scratch assay, and their survival, as revealed by the decreased expression of NCAM. These extracts are also safe to expose normal cells to, since they did not exhibit any inhibitory effects in primary astrocytic and neuronal cultures. T. cordifolia may have potential as a phytotherapeutic intervention for the treatment of glioblastomas and neuroblastomas in humans, since chloroform and hexane extracts of the plant were observed to inhibit the migration of these tumours as shown in Fig. 3 [34].

Apoptosis:

Apoptosis, often termed programmed cell death, holds a pivotal role in numerous biological processes, including tissue homeostasis and the removal of dysfunctional cells to sustain normal cellular function. This process helps eliminate damaged or undesirable cells by coordinating a cascade of carefully designed cellular activities. For the immune system to work properly and tissues to remain intact, apoptosis control is crucial [35].

Anti-apoptosis effects:

Additionally, tinospora cordifolia contains anti-apoptotic properties. T. cordifolia has shown in a number of cellular systems that its bioactive components and extracts can alter apoptotic pathways and decrease cell death. The putative therapeutic benefits of T. cordifolia in various diseases and conditions may be supported by these anti-apoptotic actions [36].

In a study conducted by Sharma *et al.*, in 2018, bioassay-guided fractionation was employed to investigate the anti-cancer properties of a natural chemical, specifically a clerodane furano diterpene glycoside, derived from Tinospora cordifolia. Various cancer cell lines, including A549, PC-3, SF-269, MDA-MB-435, HCT-116, and MCF-7, were utilized in the experiments. Preliminary screening assays revealed that the PC-3, MDA-MB-435, and HCT-116 cancer cell lines exhibited the highest responsiveness to the compound TC-2 derived from T. cordifolia. In particular, HCT-116 cells showed an upregulation of ROS and oxidative stress (OS) after TC-2 treatment. Also, TC-2 induced mitochondria-mediated apoptosis and autophagy in HCT-116 cells, which showed anticancer effectiveness. Apoptosis was confirmed by double staining with Annexin-V FITC and propidium iodide (PI), which revealed the replacement of phosphatidylserine after 24 hours, indicating the occurrence of cell death. These findings suggest that the clerodane furano diterpene glycoside derived from Tinospora cordifolia exhibits potential anti-cancer effects, particularly in the PC-3, MDA-MB-435, and HCT-116 cancer cell lines [4]. Using a hexane extract fraction of T. cordifolia (TcHf) matured stems, *Thippeswamy et al. (2007)* investigated the action of TcHf on Ehrlich ascites tumour (EAT) in mice. The antiproliferative (inhibitory) impact of TcHf on EAT cells was seen in vivo at a dose of 100µl. TcHf

allowed for the identification and prevention of apoptosis, apoptotic bodies, nuclear condensation, the characteristic DNA ladder, caspase-3 activation, and a decrease in cell number and size in EAT. TcHf also inhibited cell cycle progression in the G1 phase. Not only did TcHf increase Bax expression, but it decreased Bcl-2 expression as well [37].

Table 1.1: Anti-cancer activities and molecular mechanism of *T.cardifolia*

Sr no.	Type of cancer	Cell Line	Experimental Protocol	Plant source	Solvent	Molecular target	IC_{50}	Reference
1	**Colon cancer** (vitro)	Adenocarcinoma HCA-7	Cell viability test, RNA isolation, reverse transcription, and quantitative RT-PCR	Dried powder of Stem	2L of MeOH/H2O (1:1)	Diff ⊥ Proliferation ⊥	------	[14]
2	**Oral cancer** (Vivo)	AW13516	MTT assay for measuring cell viability, flow cytometry for measuring mitochondrial potential, and an annexin-V/PI assay for measuring apoptosis	Stem powder	distilled water	Apoptosis mediated cell death ↑	------	[15]
3	Lung cancer (Vivo)	Male BALB/c mice (25-30 g)	Glutathione-S-transferase (GST) UDP-Glucouranosyl-transferase (UDP-GT) Reduced Glutathione (GSH)	stem extract	distilled water	Glutathione ↓ and lipid peroxidation levels↓	------	[10]
4	Lung cancer (Vitro)	adenocarcinoma cell line A549	Passaging of cells, MTT assay, Trypan blue dye exclusion assay, Mitochondrial membrane potential determination, Cytomorphological changes in A549 cells	Leaves	Water	--------	IC50 concentration is 100 ppm	[16]

5	Breast cancer (vitro)	MDA-MB-231, MCF-7, HACAT cells	Cell viability assay, Clonogenicity assay, Hoechst s staining, MTT assay	Stem	hexane-chloroform-ethyl acetate and methanol	Chloroform fraction =35.6±3.66 (MDA-MB-231 Cells),28.9±2.93(MCF- 7 Cells) 120.1±3.16(HACAT Cells)	ROS↑, p21↑, p53↑, Bax↑, Bad↑, Bcl2↓, c-myc↓, PARP↓, caspase-3↑	[17]
6	Triple negative breast cancer(TNBC) (vivo,vitro)	MDA-MB-231	immunoblotting, DNA fragmentation assay, annexin-V staining, Cell cycle analysis	leaves	ethyl acetate and methanol (2:1, v/v), and 6:2:5:2, v/v(toluene, ethyl acetate, chloroform, and acetic acid)	------	ROS↑, intracellular calcium levels↑, ΔΨm↓, MPTP↓, cardiolipin peroxidation↓, cytochrome c↓,, phosphorylation of p53↑, Bcl-2 ↓, Bax ↓, G1 population↑, cleaved caspase-3↓, proliferation⊥	[19]
7	Protective effects of ethanolic Extract of Tinospora cordifolia for differentiation-inducing in Glioblastomas	C6 glioma,	cell cycle analysis, Wound scratch assay, MTT assay	stem	hexane, chloroform, ethyl acetate, and butanol	proliferation↓, NCAM ↓, PSA-NCAM↓,MMP-2 and 9↓,D1⊥ ,Bcl-xL ⊥ , HSP70↑,CDKN2A↓	250 µg/ml(C6, U87MG and HeLa cells)450 µg/ml(PC3 cells)	[22]

8	Alcoholic extract effects on proliferation and differentiation of Tinospora cordifolia on proliferation and differentiation of bone marrow cells in the host having a tumor (vivo, vitro)	L929,, BMDM	MTT assay, Assay of TNF activity, Assay for nitrite production, Assay of IL-1 activity	Whole plant	70% EtOH	IL-1↑, TNF↑, NO↑, CFU-GM↑	------	[23]
9	Effect of alcoholic extract of Tinospora Cordifolia on Dendritic Cells (vitro, vivo)	Dendritic cells (DC)	MTT (Tetrazolium) Assay, Cytotoxicity Assay,	Stem of plan	70% ethanol	IL-1↑, TNF-α↑, NO↑	------	[24]
10	tumor angiogenesis (vivo)	MDA-MB-231 cells(Mice)	Trypan blue dye exclusion assay, MTT assay, Rat corneal micro-pocket assay	leaves	a mixture of ethyl acetate and methanol in a ratio of 2:1 (v/v)	VEGF-induced cell migration⊥, Poliferation⊥	IC50 value for TCCP in MDA-MB-231 cells was determined to be 68.2 mM	[27]
11	breast cancer	MDA-MB-231	Rat corneal angiogenic assay, Endothelial cell tube formation assay (in vitro)	stems	Hexane, benzene, chloroform, ethyl acetate, and methanol	p38 MAP kinase regulates VEGF gene expression ⊥	------	[28]
12	Hepatocellular carcinoma (HCC) (Vivo)	Rats with hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DEN)	Behavioral and toxicological effects, Histopathological study	Stems (Epoxy clerodane diterpene) compound used	Alcohol extraction	methionine aminopeptidase ⊥, production of interleukin-8 (IL-8) ⊥	----	[30]

13	Human cervical cancer cells(proliferative) [vivo and in vitro]	HeLa cell line	Cell viability by MTT assay, SRB assay	stems	Ethanol, petroleum ether, chloromethane, n-butanol	Proliferation ⊥	The IC50 values of extracts were found within the range of 2.57 ± 0.31– 183.47 ± 2.20 µg/mL	[33]
14	Brain cancer (Vitro)	U87MG glioblastoma and IMR-32 neuroblastoma cell lines	Immunostaining, Wound scratch assay	stem	ethanol	proliferation and migration ↓, induces in differenciation	25 µg/mL and 50 µg/mL for Chl-TCE and 32.5 µg/mL and 35 µg/mL for Hex-TCE	[34]
15	Generation of autophagy and apoptosis by t.cordifolia natural compound in colon cancer cells (in vitro)	HCT-116,A549,PC-3, SF-269, MDA-MB-435 ,MCF-7	spectroscopic data interpretation, single-crystal X-ray crystallographic analysis, SRB assay, immunofluorescence microscopy, bioassay-guided fractionation	Fresh stems	4.5 L of ethyl acetate: water in the ratio of 1:1	ROS↑	------	[4]
16	Effects of hexane fraction of Tinospora cordifolia in EAT cells (vivo)	Ehrlich ascites tumor (EAT)	Western blot analysis	matured stems	hexane–benzene–chloroform–ethyl acetate, methanol	proliferation⊥, Bax↑, Bcl-2↓, activation of caspase-3	------	[37]

Inflammation:

Inflammation represents the tissue's response to injury, intended to eradicate the source of damage and foster the healing process. An integral function of an inflammatory reaction is to serve as an initial defense mechanism against infections. The observable clinical manifestations of acute inflammation, including redness, swelling, heat, and pain, have been acknowledged since ancient times [38].

Anti-inflammatory activity:

Tinospora cordifolia (TC) has outstanding anti-inflammatory qualities and is helpful to cure inflammatory illnesses [39]. Inflammation can be characterised as a complex immune system reaction used to resist the effects of harmful stimuli, such as the development of tumours, damaged cells, poisons, pathogenic microorganisms, and irritants. Despite the possibility of considerable differences in the type, nature, and inflammatory responses across various illness states, they are primarily identified by the irregular adjustment of a typical kind of genes. It is impractical to concentrate treatment efforts on any one specific region since an inflammatory reaction necessitates a variety of multiple-signaling pathways and mediators as it transmits inflammatory impulses to different tissues and cell types. As a result, controlling the production or function of cytokines is a valuable method for reducing inflammatory responses [40, 41]. Due to its link with practically all types of diseases that affect mortal creatures, inflammation has captured the interest of the scientific study community [42].

Ramadhan *et al.*, (2021) conducted a research in which they were to determine out how Silver Nanoparticles (AgNPs) affect the liver function of male rats and examine if Tinospora cordifolia (T.C.) could protect against AgNPs-induced liver diseases. Rats were divided into four groups: control, AgNPs-treated, AgNPs + T.C, and T.C-treated. Liver enzyme levels—alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—were assessed to identify liver function irregularities. The AgNPs-treated group exhibited escalated liver enzyme activity compared to the control, indicating AgNPs-induced liver dysfunction. However, co-administration of T.C with AgNPs notably mitigated liver enzyme levels, signifying T.C's protective impact against AgNPs-induced liver dysfunction. Another study corroborated T.C extract's hepatoprotective effect against gold nanoparticles' toxicity in male rats. T.C extract safeguarded hepatocytes from damage and curbed elevated liver enzymes associated with impaired liver function. The findings underscore AgNPs' detrimental influence on liver function, as illustrated in Table 1.2. Nonetheless, Tinospora cordifolia extract emerges as an effective shield against AgNPs-induced liver dysfunction in rat models [43].

In another study, Philip, S *et al.*, (2021) was to investigate the potential anti-inflammatory effects of a chloroform extract of Tinospora cordifolia (CETC) by using both in vitro and in vivo models. By limiting the nuclear translocation of NF-κB, CETC dramatically lowered the levels of pro-inflammatory biomarkers in THP-1 cells. The in vivo study showed that pre-treatment with CETC improved the survival rate in rats during endotoxemic episodes and suppressed the tissue expression of pro-inflammatory proteins. These findings provide insights into the anti-inflammatory mechanism of CETC and support its potential as a therapeutic agent for disorders associated with dysregulated immune reactions. Further research is warranted to explore the clinical applications of CETC in treating inflammatory conditions and develop drugs targeting deregulated immune responses [39]. *Ghatpande, N. S. et al., (2019)* investigated the systemic disruption of iron balance linked with inflammation-driven anemia (AI), primarily attributed to the heightened expression of hepcidin. Tinospora cordifolia (TC) has demonstrated notable anti-inflammatory properties, suggesting potential efficacy in managing inflammatory conditions. They conducted in vivo and in vitro experiments to assess TC's impact on AI, coupled with HPLC studies aimed at identifying TC extract's active constituents. The TC-treated groups exhibited markedly elevated levels of hemoglobin (Hb) and red blood cell (RBC) count compared to the inflammatory control group. TC treatment correlated with reduced expression of the HAMP gene in rat livers, restrained inflammatory cytokines, and diminished nitric oxide (NO) production. These findings highlight the effects of TC as a therapeutic approach for managing AI and suggest its role in maintaining iron homeostasis through modulation of inflammatory responses. Further research is warranted to elucidate the underlying mechanisms of TC's anti-inflammatory effects and its potential clinical applications in AI management [44].

Anti-oxidant activity:

Oxidative stress is the term used to describe the imbalance in development which is caused by reactive oxygen and nitrogen species as well as the ability of the body's antioxidant system to counteract them and repair the damage. Proteins, nucleic acids, and lipids can change when oxidative stress levels are high. Oxidative stress can be the leading cause of mitochondrial function loss and cell death. Hydroxyl radicals, hydrogen peroxide, and superoxide anions can also make people ill with conditions like diabetes, hypertension, rheumatoid arthritis, Alzheimer's disease, and cancer [45]. By blocking the oxidation pathway and providing hydrogen, Antioxidants can also halt chain reactions [46].

A critical function in limiting tissue damage brought on by oxidative stress and searching for or stopping the growth of reactive oxygen species. Endogenous antioxidants maintain a reducing state in cells while they are in a physiological state. These endogenous antioxidant complexes don't always act well enough. As a result, one of the most sustainable approaches in modern therapy—supplementing from medicinal plants that naturally contain biomolecules—might prevent the oxidative cellular damage brought on by ROS [13]. Because it contains antioxidants like polyphenols, tinospora cordifolia extract has been employed as a naturally occurring phytochemical antioxidant medicinal treatment [47]. Numerous chemical components, including phenolic compounds, alkaloids, glycosides, sesquiterpenoids, steroids, aliphatic compounds, and diterpenoid lactones that are involved in immunomodulation processes, are linked to Tinospora Cordifolia's beneficial characteristics [48].

In a study by González-Masís *et al.*, (2020), the anti-oxidant properties of Tinospora cordifolia (TC) were investigated. The effects on the survival of mouse 3T3 fibroblast cells and cutaneous irritation tests in New Zealand rabbits were assessed after the development of a formulation of aqueous nanoparticles with concentrations of 2.5, 25, and 250 g/mL (in vivo) and 12.45 mg/mL (in vitro). No major changes that might threaten animals' health were detected in the results. These results provide more evidence that TC nanoparticles may be useful as an antioxidant without causing any detrimental side effects. Further research is warranted to explore the mechanisms underlying the anti-oxidant properties of TC and its potential applications in oxidative stress-related disorders [47]. To investigate the antioxidant potential of Tinospora cordifolia (TC), a study conducted by Prince, P.S. *et al.*, (1999) in which an oral administration of an aqueous TC root extract (TCREt) was conducted in rats. The rats were administered a dosage of 5.0 grams per kilogram of

TCREt, and researchers examined the impact of TCREt on markers related to antioxidant activity. The findings indicated a decline in ceruloplasmin and α tocopherol plasma reactive substances suggesting a reduction in oxidative stress. Moreover, there was an elevation in vitamin C and glutathione levels, which are antioxidants, within the body. Furthermore, the administration of TCREt resulted in a reduction in alloxan-induced diabetes in the rats. Alloxan is a compound known to induce oxidative stress and damage pancreatic β-cells, leading to diabetes. The observed effects of TCREt suggest its potential as an antioxidant agent in combating oxidative stress-related disorders and providing protection against diabetes [49].

To evaluate the antioxidant properties of Tinospora cordifolia (TC), Jayaprakash, R. *et al.,* (2015) conducted a study in which male Wistar albino rats were given a TC extract at a dose of 300 mg/kg body weight. The study aimed to assess how TC affected the levels of both enzymatic antioxidants as well as lipid peroxidation (LPO), in rats with liver cancer. The results revealed that liver cancer-bearing rats exhibited increased LPO levels, indicating stress, along with reduced levels of non-enzymatic antioxidants. However, when the rats were treated with the TC extract, LPO levels decreased, suggesting a reduction in oxidative damage, as shown in Table 1.2. Moreover, the levels of non-enzymatic antioxidants were restored to nearly normal levels. These findings indicate that Tinospora cordifolia exhibits antioxidant activity by reducing LPO levels and restoring the defense system in rats, with liver cancer as shown in Table 1.2 [50].

Table 1.2: Anti oxidant and anti Inflammatory activities of *T.cardifolia*

Sr No.	Experimental Protocol	Tested plant material	Extract	Organism tested	Dose/Conc.	Molecular Target	Reference
1	biochemical analyzes, statistical assay, and use of silver Nanoparticles with Tinospora Cordifolia	Whole plant	GidhuchiPure Extract	Male rats (in vivo)	400 mg/kg	ALT ↓, AST↓, ALP↓	[43]
2	laser scanning confocal microscopy, Western blot examination, determination of nitrite, PGE2, IL-1, and IL-6 levels in cell culture supernatant were measured by ELISA, test for cell viability, histological examination, ELISA measurement of TNF- and IL-1 in rat blood, TNF-, COX-2, and iNOS immunohistochemical examination of the liver, lungs, heart, and kidneys, Analysis of CETC using LC/MS Statistic evaluation	Whole plant	chloroform	male Charles Wistar rats (in vivo), THP-1 cells (in vitro)	In vivo, at 250 mg/Kg and 500 mg/Kg, respectively, and 100 ng/ml In vitro	IL-1β↓, IL-6↓, iNOS↓, COX-2↓ and ⊥ IF-α↓, (In vivo), LPS inducible cytokines ↓ (in vitro)	[39]
3	HPLC analysis, Gene expression studies, biochemical and histological analysis, qRT-PCR analysis, Nitric oxide estimation	Whole plant	Aqueous	Male Wistar rats (in vivo), RAW 264.7 cells (in vitro)	400 mg/kg BW (in vivo), 100 – 500 Mg/ml (in vitro)	Gene expression of TNF-α and Cox-2↓, gene expression of HAMP and TLR-4, Hb level↑, RBC count↑ liver and spleen iron↑ (in vivo), LPS induced NO production↓, gene expression of HAMP↓, interleukin-1beta (IL-1β) (in vitro)	[44]

4	Viability Assay, dermal irritability tests, Fourier-Transform Infrared Spectroscopy (FTIR), (MALDI-ToF MS), Nuclear Magnetic Resonance Spectroscopy (NMR), Total Polyphenol Content, Cell Culture, Atomic Force Microscopy (AFM), and Dynamic Light Scattering (DLS).	stems	Nanoparticle Aqueous Formulation.	New Zealand rabbit (in vivo), mouse 3T3 fibroblast cell (in vitro)	12.45 mg/mL (in vivo), 2.5,25 and 250 µg/mL (in vitro)	--------	[47]
5	Determination of thiobarbituric acid reactive substance, Estimation of a-tocopherol and reduced glutathione, Estimation of ceruloplasmin and vitamin C, Statistical analysis	Roots	Aqueous	Rats (in vivo)	5.0 g/kg	Plasma thiobarbituric reactive substances↓, α-tocopherol↓, Ceruplasmin↓,GSH↑, Vitamin C↑	[49]
6	Assay of enzymic antioxidants, Assay of nonenzymic antioxidants, Statistical analysis	whole plant	ethanolic	male Wister albino rats	300 mg/kg body weight	lipid peroxidation (LPO)↓, SOD↑, CAT↑, GPx↑ GSH↑, Vitamin C↑, Vitamin E↑	[50]

Anti-microbial effects:

For ages, people have encountered illnesses caused by bacteria, fungi, and other types of germs. Multiple medicines have been discovered like antibiotics and chemicals, but numerous exceptions are danger for human fitness like drug resistance. Therefore, the spread and uplift of anti-microbial materials have flourished in the therapy of diseases [51]. Plant extracts and oils have several anti-microbial activities, which have been accepted for several ages [52]. The extracts of Tinospora cordifolia have several antibacterial activities [53]. *Agarwal et al. (2019)* examined the antibacterial efficacy of powdered t.cordifolia leaves against Streptococcus mutans. Streptococcus mutans was combated using T.cordifolia in seven different concentrations. The maximum antibacterial activity of T. cordifolia was detected in a zone of inhibition measuring 19 mm in diameter at 2% concentration in a volume of 40µl. With a volume of 30 µl and a chlorhexidine concentration of 0.2%, a 28 mm zone of inhibition was seen. In contrast to S.mutans, T.cordifolia showed antibacterial action [53]. Rai, A. *et al.,* (2015) conducted research in which their aim was to examine and compare the ability of four extracts: Tulsi (sanctum), Harad (Terminalia chebula), Guduchi (Tinospora cordifolia), and Licorice (Glycyrrhiza glabra), to prevent tooth decay. The study involves testing these extracts against two types of bacteria known to contribute to tooth decay: Streptococcus mutans and Lactobacillus acidophilus. Additionally, the research seeks to analyze the depth of decay caused by these extracts and compare their effectiveness. The results of phase I demonstrated that Glycyrrhiza glabra (Licorice) extract exhibited the highest zone of inhibition against the tested bacteria, followed by Ocimum sanctum (Tulsi), Terminalia chebula (Harad), and Tinospora cordifolia (Guduchi), respectively. In phase II, it was observed that Tinospora cordifolia (Guduchi) extract resulted in the highest decay depth, followed by Terminalia chebula (Harad), Ocimum sanctum (Tulsi), and Glycyrrhiza glabra (Licorice). In conclusion, the extract of Glycyrrhiza glabra (Licorice) demonstrated high effectiveness against Streptococcus mutans and Lactobacillus acidophilus. These results emphasize the potential of extracts, in preventing tooth decay and encourage research into how they work against cariogenic microorganisms as illustrated in Table 1.3 [54].

In a different study, *Molla et al., (2012)* examined compounds, t. cordifolia's antibacterial properties, and lethal extracts from the plant's fresh stems. Compound TC-1 had substantial antibacterial and antifungal activity against microorganisms examined using the "Disc diffusion method." Minimum Inhibition by TC-1 against Bacillus megaterium and Salmonella was at a 128 g/ml concentration. At the same time, the lethal concentration of TC-1 was 9.34 g/ml. It might be said that the compound TC-1 is an alkaloid with noticeable effect [55]. Prajwala B *et al.,* 2018 evaluated antibacterial efficacy against Escherichia coli cell division in this investigation. T. cordifolia was shown to include alkaloids, carbohydrates, terpenoids, steroids,

tannins, amino acids, flavonoids, and glycosides by assay for phytochemicals screening. HPLC analysis showed that t.cordifolia contained berberine as well. Methanolic extract has the highest concentration of phytochemicals, while chloroform, hexane, and acetone extracts have the lowest concentrations. In contrast, the extract that significantly reduced bacterial growth and indicated an E. coli inhibitory zone was the methanol extract [56].

Table 1.3: Anti microbial activities of *T.cardifolia*

Sr No.	Model used	Plant source	Experimental protocol	Solvent	Target microorganism	Cell line	Study Design	Result	Reference
1	Anti-microbial activity of Tinospora cordifolia against Streptococcus mutans	Leaves powder	descriptive analytic tests, maceration	100% Ethanol	Streptococcus mutans	-----	In vitro	19mm of inhibition at 2% with volume of 40 µl, 28 mm of inhibition at 0.2% of chlorhexidine with volume of 30 µl.	[53]
2	Anti-microbial activity of Four Different Plants including T.cordifolia against Cariogenic Bacteria	Leaves powder	One-way Kruskal–Wallis test, one-way ANOVA test	Ethanol, Methanol, and Water	Lactobacillus acidophilus, Streptococcus mutans	ATCC0885, ATCC 25175	In vitro	Inhibitory effects against S. mutans and L. acidophilus.	[54]
3	Inspection of chemicals and microbial activity of T. t.cordifolia	Fresh stem	standard chromatographic techniques, crystallization, FTIR, 1H NMR, 13C NMR spectral analyses, Disc diffusion method, Serial Dilution Technique	Rectified spirit	Brine shrimp nauplii., Bacillus megaterium (Gram positive bacteria) Salmonella typhi-A(Gram negative bacteria)	----	In vitro	At 128µg/ml, t.cordifolia shows minimum inhibition against megaterium and Salmonella typhi-A, whereas lethal concentration is 9.34 µg/ml.	[55]
4	Anti-bacterial activity of Tinospora cordifolia against Escherichia coli and its phytochemical screening	Leaves powder	Phytochemical screening assay, HPLC analysis, disc diffusion method	Methanol, Ampicillin, Ethanol, Chloroform, Hexane, Acetone	Escherichia coli	------	In vitro	Methanolic extract of t.cordifolia is involved in inhibition	[56]

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