Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 1039 - 1056

Review Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MEDICINAL PROPERTIES AND GENETIC DIVERSITY OF A MAGICAL SHRUB *TINOSPORA CARDIFOLIA*: AN UPDATE

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ABSTRACT

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines. Medicinal plants were the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. *Tinospora cardifolia* is one of the important medicinal plants which is a large and deciduous climbing shrub found at higher altitudes of India cultivated in tropical areas of India and known by names such as Gurcha, Heart leaved moonseed, Heavenly elixir and Jetwatika. About 152 plants have been documented in India for traditional ayurvedic medicines and out of theses 152 plants; *T. cardifolia* is one of the most promising plants in the herbal medicine system. The Plant *Tinospora* includes 76 scientific rank species but only 13 of these species are accepted for medicinal uses. Different parts of *T. cardifolia* such as leaves, roots and stem are emetic and used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections such as rheumatism, urinary diseases, dyspepsia, general debility, syphilis, skin diseases, bronchitis, spermatorrhea, impotence, general debility, dyspepsia, fevers, urinary diseases, visceral obstruction and leprosy. This review gives a bird view of the main biological activities, pharmacological actions, medicinal applications of *T. cardifolia* extracts and genetic diversity of *T. cardifolia* .

KEY WORDS: ***Tinospora cardifolia,*** **antimicrobial, genetic diversity, medicinal, Rasayanas.**

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INTRODUCTION

The tradition to use plant as a medicine is thousands of years old. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments[1]. Many drugs commonly used today are having herbal origin. Some are made from plant extracts while others are synthesized to mimic a natural plant compound. In recent years, pharmaceutical companies were spending a lot of time and money in developing natural products that are extracted from plants. Different parts of the world are working to produce more cost effective remedies that are affordable to the public[2]. In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases and physiological abnormalities under practice such as Ayurveda, Siddha and Unani. Research in herbal medicine has recently been revolutionized with the identification of several botanical plants with established physiological effect and efficacy for clinical condition either alone or combination with pharmaceuticals. Ayurveda was a comprehensive natural health care system that organized in India more than 5000 years ago. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid, tocopherols, polyphenolic compounds and terpenoids. They perform several important functions in plants and humans[3]. *T. cardifolia* commonly named as "Guduchi" is known for its immense application in the treatment of various diseases in the traditional ayurvedic literature[4]. In India, various extracts of *T. cardifolia* are used as a remedy for many diseases and are included in various polyherbal preparations used for the treatment of diabetes, hepatitis, etc. During the last few decades considerable progress has been achieved regarding its biological activity and medicinal applications. Hence, it can be chosen as a source for the development of industrial products for treatment of various diseases. This review gives a bird view of the main biological activities, pharmacological actions, medicinal applications of *T. cardifolia* extracts and also biological activities of few *T. cardifolia* compounds isolated[5].

Medicinal properties of Tinospora cardifolia

Ayurvedic literature quoted *T. cardifolia* as a constituent of several compound preparations which has been used in general debility, dyspepsia, fever and urinary diseases[6]. *T. cardifolia* family is rich in various compounds i.e simple sugars, rhamnose, glucosinolates and isothiocyanates[7]. It has multiple actions like stem is a bitter stomachic i.e it strengthens and excites stomach activities and stimulates bile secretion also[8]. Fresh juice of guduchi, when mixed with rock candy, speeds up the recovery in hepatitis patients and leaves of *T. cardifolia* along with honey acts as a tonic, enriches the blood and enlarges the spleen[9]. The juice of the plant is useful in diabetes, vaginal and urethral discharges and root and stem are prescribed in combination with other drugs as an antidote to snake bite and scorpion sting[10]. The herb accords longevity, enhances memory, improves health, bestows youth, betters complexion, voice, energy and luster of the skin and hence enjoyed the reputation among ancient Hindu writers of being an aphrodisiac[11]. Guduchi juice, when taken with cow's milk or lodhra, is effective in combating leucorrhea and the juice when consumed after mixing with cumin seeds reduces the burning sensation caused due to pitta[12].

BOTANICAL AND PHARMACOGNOSTICAL PROPERTIES

Botanical description

The plant is large, deciduous extensively climbing shrub with several elongated twining branches. Leaves are simple, exstipulate, long petioles upto 15 cm. Both at base and apex they are roundish and pulvinate. Flowers grow separately on small plants and appear only when the plant is leafless, greenish yellow on axillary and terminal racemes. Sepals are 6 in number and smaller ones are on outer side while inner ones are on the inner side. Petals are membranous, smaller than sepals and are obvate. Fruits appear in aggregate of 1-3, ovoid

smooth druplets on thick stalk with sub terminal style scars, scarlet or orange coloured[13].

Ethanobotanical and Pharmacognostical description

Various parts of *T. cardifolia* are being prescribed in Ayurveda and other systems of medicine as a monoherbal or polyherbal preparation[14]. Since each part of *T. cardifolia* has some medicinal property, it is very much commercially exploitable. The diagnostic pharmacognostical characteristics of medicinal parts are as follows.

Leaf

Single layer of epidermis and a wide zone of cortex composed of 3-4 layers of epidermis are found in cross section of leaf. Trichomes are not found. Petiole is circular in the tranverse section. The vascular bundles consist of radial rows of cambium cells on the outer side and a few rows of cambium cells on the outer side followed by phloem[15]. The epidermal cells surround the base. Starch is present throughout the tissue[16]. Veins are multicostate and prominent on dorsal side[17,18]. Leaves of *T. cardifolia* acts as Vasorelaxant and relaxes norepinephrine induced contractions[19] and inhibits Ca++influx[20,21]. Leaves acts as anti-inflammatory, anti-microbial, antihypertensive and anti-viral agents[22]. Leaves of *T. cardifolia* induce apoptosis in leukemia by activating caspase-3and bax[23].

Stem

Stems are greyish green with smooth surfaces and swelling at nodes, older ones are light brown and surface marked with warty protuberances due to circular lenticels. Outer bark is thin and papery brown to greyish in colour[24]. Lenticels are circular and prominent. Transverse surface have wheel like structure which is a characteristic feature of family *Menispermaceae*[25]. Stems of *T. cardifolia* are useful in the Treatment of neurological disorders like Parkinson[26], Dementia[27], motor and cognitive deficit, neuron loss in spine and hypothalamus[28]. Stems by Immunomodulation inhibit NF-kBand[29] and acts as nitric oxide scavenger to show anticancer activities[30].

Root

The young aerial roots are long filiform, threadlike, squairsh, which arise from the mature branches or cut bits of stems grow downward and by continuously lengthening sometimes reach the ground and these roots are characterized by tenta to penta primary structure[31]. Oval or elliptical starch grains are present which are mostly simple but sometimes as compound grains of 2-5 components with the presence of central hilum and concentric striations[32]. Roots are useful in anti-viral infections[33], anticancer[34] and anti-diabetes[35,36]. Roots are helpful in the treatment of inflammation[37], Neurological and psychiatric conditions[38]. Roots acts as Protease inhibitors for HIV and drug resistant HIV[39,40].

Flowers

Flowers are unisexual, racemes, greenish yellow in colour, appears when plant is leaf less. Male flowers are clustered together but female flowers exist in solitary inflorescence[41]. Flowering occurs during March to June[42]; Flowers were useful in the treatment of IgA neuropathy and glucocorticoid induced osteoporosis which is in the early stage of inflammatory arthritis[43]. Flowers also induce cell cycle arrest in G2/M phase and apoptosis through c-Myc suppression[44]. Flowers are useful in the Inhibition of TNF-α, IL-1 β, IL-6 and COX-2[45].

Fruits

Fruits of *T. cardifolia* grow in aggregates of 1-3 ovoid smooth drupelets on thick stalk with sub terminal style scars. Colour of the fruits is Scarlet or orange red color. Fruits are found during winter season[46]. Fruit was a source of bioactive food colorants[47]. The mature fruits are closely associated with cell metabolism[48] and growth[49,50].

CHEMICAL CONSTITUENTS

Phytochemicals are the chemicals produced by plants. These refer to those chemicals which may have an impact on health, or on flavour, texture, smell or color of the plants, but are not required by humans as essential nutrients. In plants, phytochemicals are the

secondary metabolites such as alkaloids, terpenoids, steroids, tannins, phenolic compounds, flavonoids, resins fatty acids and gums which are capable of producing definite physiological action on body[51].

Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. *T. cardifolia* is rich in various alkaloids such as Berberine, Palmatine, Tembetarine, Magnoflorine, Choline, Tinosporin[52,53,54,55,56,57], Isocolumbin , Palmetine, Tetrahydropalmatine, Magnoflorine[58], Aporphine, Jatrorrhizine and Tetrahydropalmatine[52,53,54,55,56]. Alkaloids of *T. cardifolia* possess antitumor activity and are also used as medications or as recreational drug in various pharmacological conditions.

Diterpenoids

Diterpenoids present in *T. cardifolia* are Furanolactone Lactones and Clerodane derivatives[59] **,** 5R,10R-4R-8R-dihydroxy-2S-3R:15,16-diepoxy-cleroda-13 (16), 14-dieno-17,12S:18,1S-dilactone[52,53,54,55,56,57]. These terpenoids have medicinal properties such as anti- carcinogenic, antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial and diuretic (e.g. glycyrrhizin) activity. Terpenoids and their derivatives also belong to other drugs such as validol, menovasin, turpentine, bromkamfora etc.[60].

Steroids

Steroids of *T. cardifolia* are β–sitosterol, δ-sitosterol, alpha hydroxyacetone, 20 β-hydroxyecdysone, Ecdysterone, Makisterone A and Giloinsterol[44,45,61]. Steroids stimulate the growth of osteoblasts, increases the differentiation of cells into osteoblastic lineages and also increases the mineralization of bone like matrix[4].

Glycosides

Glycosides present in *T. cardifolia* are 18-norclerodane glucoside, Furanoid diterpene glucoside[26,29,62,] Tinocordiside, Tinocordifolioside, Cordioside, Cordifolioside A, Cordifolioside B, Syringin, Syringin-apiosylglycoside, Palmatosides C, Palmatosides F, Cordifoliside A, Cordiofoliside B, Cordifoliside C, Cordifoliside D and Cordifoliside E and Pregnane glycoside[21,26,29,63]. These all glycosides have many properties such as antioxidant or radical scavenging activity, inhibition of lipid peroxidation and lipoxygenases *in vitro*[64], antimicrobial and antiviral[65], antimutagenic[66], and antidiabetic properties[67]. The antioxidant activity of glycosides provides synergistic benefits for the treatment of diabetes because of their insulin enhancing potential[68].

Sesquiterpenoid

Tinocordifolin is an important sesquiterpenoid present in *T. cardifolia* which plays a major role in plant development. They act as constitutive protective agents against invading organisms, signal molecules and allelopathic compounds and natural animal toxicants. A regular intake of Sesquiterpenoid decreases the incidence of certain forms of cancer so they were known as chemo-preventive agents[69].

Miscillaneous compounds

Other compounds in *T. cardifolia* which are immunologically active are Giloin, Giloinin, Tinosporic acid, Tinospororan acetate, Tinosporal acetate, Tinosporidine, Aliphatic Octacosanol, Heptacosanol compound, Nonacosan, Heptacosanol, Cordifolane, Octacosanol, Tinosporic acid, Tinosponone, Tinosporal, 2-Phytoecdysone and Arabinogalactan[39,40]. Tinosporic acid and Tinospororan acetate showed protection against aflatoxin induced nephratoxin[37]. Cordifolane, Arabinogalactan and Giloinin showed radio protective effects[122].

GENETIC DIVERSITY

The genetic diversity of a species is an important element in establishing conservation programs because the ability of a species to respond adaptively to environmental changes depends on the level of genetic variability it contains[70,71,72]. Therefore, assessing the level and distribution of genetic diversity are crucial for management and the development of effective conservation strategies. Faced with

the problem of preserving rare and endangered species, great concerns also should be concentrated on the endemic species with restricted geographic distribution[73]. Medicinal plants are increasingly endangered. The survival index of a species is greatly determined by the percentage of polymorphism and the gene flow between the populations. Molecular markers like RAPD, AFLP, RFLP, SSR SCAR etc are being continuously used for assessing the genetic diversity of plants and these are considered vital for formulating conservation strategies for endangered medicinal plants[74]. In order to develop an efficient identification method, molecular techniques have been used since these are reliable, unaffected by environmental conditions and can aid varietal identification[75]. Among the different types of molecular markers available, random amplified polymorphic DNA (RAPD) is useful for the assessment of genetic diversity among rare species of medicinal plants[76]. RAPD markers have many advantages like the requirement of very small quantity of template DNA and no involvement of radioactive or hybridization techniques. RAPD markers have been extensively used in constructing linkage maps, assessment of genetic variation in population, gene tagging and identification of cultivars and species[77]. Molecular markers are not tissue-specific and can be detected at any stage of plant development. Therefore, DNA-based authentication of medicinal plants can be a useful tool for quality control and safety monitoring of herbal pharmaceuticals and neutraceuticals. Among the polymerase chain reaction (PCR)-based DNA techniques, random amplified polymorphic DNA (RAPD) is easy to perform and requires no information about the DNA sequence to be amplified. It has been used, therefore, to study the genetic diversity in various plant species[78,79] , authenticate the herbal medicinal materials[80], and detect adulterants[81]. Rasayana churna includes three herbal drugs in powdered Dried stem of *T. cordifolia*, dried fruit of *E. officinalis* and dried fruit of *T. terestris* were collected from the Pune regions of India. After 120 primers were screened, primer OPA-6 was screened and selected to identify *T.cordifolia, E. officinalis* and *T. terestris* in Ayurvedic prescription[82]. Identified germplasm is an important component for the efficient and effective management of plant genetic resources. Studies were undertaken for identification and to assess genetic variation within 15 clones of *T. cardifolia* collected from Bhubaneswar through random amplified polymorphic DNA (RAPD) markers. Analysis was made using forty decamer primers. Out of them, 15 primers were selected and used for identification and genetic relationships within 15 clones. The genetic distance was very close within the clones. This polymorphism study with the help of RAPD markers will be helpful to know the genetic background of the medicinal plants with high commercial value and also provides a major input into conservation biology[83]. The phylogenetic relationships among the three species of *Tinospora* found in India are poorly understood. Morphology alone does not fully help to resolve the phylogeny and therefore a fast approach used molecular analysis was explored. Two molecular approaches viz Random Amplified Polymorphic DNA (RAPD) assay and restriction digestion of ITS1-5.8S-ITS2 rDNA Restriction fragment length polymorphism (RFLP) were used to evaluated the genetic similarities between 40 different accessions of *T. cordifolia, T. malabarica* and *T. crispa* collected from Jammu and Kashmir, Assam and South Kerala respectively. Out of 38 random primers used only six generated the polymorphism, while three out of 11 restriction enzymes used gave polymorphic restriction patterns. Three independent clones of each species *T. cordifolia, T. malabarica* and *T. crispa* were sequenced. Phylogenetic relationship inferred from ITS sequences was in agreement with RAPD data[84]. Based on morphology, the species status and taxonomic affinities of three species of *Tinospora* (*T. cordifolia*, *T. sinensis,* and *T. crispa*) with ranges in India have been questioned. To evaluate species delimitation and population structure among 40 accessions of the three species, a relatively new marker, cytochrome P450, was used. 40 accessions were collected from multiple locations within India i.e Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh,

Assam, Kerala, Haryana and Delhi. Five out of nine primers generated polymorphisms with 39 out of 47 bands were found to be polymorphic. The resulting phylogenies highly support the Indian *Tinospora* species as part of a clade (expanded Tinosporeae), consists of diverse Menispermaceae from around the world. The three Indian species are monophyletic and are most closely related to *Tinospora* species from Australia (*T. esiangkara* and *T. smilacina*)[85]. Genetic variation among the 20 selected samples of *T. cardifolia* from Western Himalayas using RAPD marker was done using 120 decamer oligonucleotide primers. Out of 120 primers used only four RAPD primers i.e OPA-16, OPC-5, OPC-7 and OPC-13 revealed low genetic diversity among *T. cardifolia*[86]. From Gujrat, Genetic diversity was accessed by isoenzymatic method using 25 accessions of the medicinal climber, *T. cardifolia* . Analysis of ten isozymes revealed the presence of 16 gene loci and 33 alleles in 25 accessions. The percentage of polymorphic loci (P) was 45.0**%** and mean observed number of alleles per locus (A) was 1.57. The average observed heterozygosity (Ho) and expected heterozygosity (He) were 0.443 and 0.270 respectively showed high levels of genetic variations among different accessions. The unweighted paired group method (UPGMA) dendrogram clearly depicted that the spectra of genetic diversity among various accessions of *T. cardifolia*[87].

TRADITIONAL USES IN INDIGENOUS SYSTEM OF MEDICINE

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities in the world. Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare products were described in Vedas and Bible. The local people have a long history of traditional plant usage for medicinal purposes[88] Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. The economic significance of medicinal plants stems from the fact that the number of patients suffering from chronic ailments is on the rise and drugs from medicinal plants are proving to be more effective in treating chronic ailments[89]. The three main species of *Tinospora are*: *T. cardifolia , Tinospora malabarica* and *Tinospora crispa. T. cardifolia mainly* found at tropical region of India and is widely used as a medicine in folk and ayurvedic systems. *T. cardifolia* is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. *Tinospora malabarica* found in Konkan, Karnataka, Tamilnadu, Bengal, and Orissa is mixed as adulterant or substitute with *T. cordifolia* and is also used as an immunomodulator[90].

ANTIMICROBIAL ACTIVITY OF TINOSPORA CARDIFOLIA

The antibacterial activity of the aqueous, ethanol and chloroform extracts were tested with the disc difffusion method against a number of Gram-negative and Gram-positive bacteria, the results suggested that the ethanolic extract has significant antibacterial activity, which justified the uses of *T. cardifolia* in traditional medicine in various parts of the countries to treat various infectious diseases[91]. Secondary infections are the most common cause of mortality in the immunocompromised host. In Ayurveda, *T. cardifolia, Asparagus racemosus* and *Withania somnifera* are prescribed as agents to strengthen host defence mechanisms so that immunocompromised host can fight with the secondary infections. Administration of *T. cardifolia*, *Asparagus racemosus* and *Withania somnifera* helped in faster bacteremia clearance in the rats and mice suffering from *Escherichia coli* peritonitis. Mortality due to *Staphylococcus aureus* sepsis in neutropenic

and hemisplenectomized mice was reduced in animals treated with *T. cardifolia, Asparagus racemosus* and *Withania somnifera*. *Candida albicans* sepsis induced mortality was also reduced by these plants. In view of their oral efficacy and lack of adverse effects, *T. cardifolia, Asparagus racemosus* and *Withania somnifera* show promise as useful immunomodulators[92].

USES OF T. CARDIFOLIA *IN FOLK AND TRIBAL MEDICINE OF INDIA*

T. cardifolia is widely used in veterinary folk medicine system of India for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic properties[93]. The tribal Baiga living in the interior areas of Naugarh and Chakia Block of Varanasi district, Uttar Pradesh make the paste of the stems of Guduchi and the roots of Bhatkatiaya. The tribals of Bombay and its neighbouring areas and the fisherman along the sea coast use *T. cardifolia* in the treatment of fever, jaundice, chronic diarrhea and dysentery. Similarly, the tribals of North Gujrat used the powder of root and stem bark of the plant along with milk in day to day life for the treatment of dysentery and diarrhea. This paste was used in the treatment of fever for three days. Decoction of stem is administered orally by the people of Jammu and Kashmir for the treatment of fever[94]. In dahanu forest division of Maharahtra, tribal races, viz. Agaris, Bhils, Dhodias, Khakaris, Dublas and Rimoshis uses the stem decoction with cold and hot water in morning in an empty stomach as a tonic in general debility. The muslim tribals of Rajouri, Jammu comprising Gujjar and Backwals used the plant in bone fracture[94]. The Inhabitants of Bhuvneshwar uses the warm juice of root of *T. cardifolia* orally for the treatment of high fever. In Punjab juice of leaves along with honey is used to lower the body temperature and the ear pain. In Haryana powder of *Terminalia chebula, T. cardifolia* and *Trachyspermum ammi* administered orally, along with salt in the morning for the treatment of cough. The local women in the tribes of Rajasthan use the paste of 5 seeds of *Piper nigrum* along with few leaves of *T. cardifolia* in the treatment of leucorrhoea. Inhabitants of Hisar take the juice of stem orally with honey for the treatment of asthma[95].

CHARACTERIZATION OF COMPOUNDS OF TINOSPORA CARDIFOLIA USING VARIOUS TECHNIQUES

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for a new drug discoveries because of the unmatched availability of chemical diversity[96]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. Researchers found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrhoeal, analgesic and wound healing activity were reported in the medicinal plants[97]. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds and these compounds must undergo extraction, isolation and characterization so that they can be used in drug development.

Thin layer chromatography (TLC) of Tinospora cardifolia

In the methanolic stem extract of *T. cardifolia* six molecules were reported having different Rf values but the major compound found was Tinosporoside with the Rf value of 0.35[13]. Chloroformic extract of *T. cardifolia* showed Rf values of 0.24 and 0.35 in thin layer chromatography (TLC) identity test which was characterized as tinosporoside. The solvent system consists of chloroform and methanol. The spraying reagent was anisaldehyde-sulphuric acid[98]. Quality of plants grown in tissue culture was ensured using the juice of the plants collected from various supports from the same garden and were analyzed by thin layer chromatography (TLC). A total number of 11 spots were observed. Out of 11 spots three Rf value i.e 0.192 (Tinosporaside), 0.551 (berberine) and 0.717 were found to be universally present in the plants and these are

used as chemical markers of the plant. Tinosporaside contents were found to be significantly high in *T. cardifolia*[99].

High-performance liquid chromatographic (HPLC) of Tinospora cardifolia

HPLC analysis of *T. cordifolia* showed six different phenolic acids (Benzoic acid, Cinnamic acid, Caffeic acid, Ferulic acid, Gallic acid and Tannic acid) present in varying amount in the ethanolic stem extract[100]. A high-performance liquid chromatographic (HPLC) method for the estimation of berberine in the stem of *T. cordifolia* and *Tinospora sinensis* was performed. HPLC analysis gave peaks at 24.54 min and 28.48 min, in the methanolic extract of *T. sinensis*. *T. cardifolia* and *T. sinensis* showed differences in chemical constituents. The physicochemical studies of *T. cardifolia* stem crude drug were evaluated. The phytochemical screening was carried out and the berberine alkaloid was quantified in different fractions of the extract by HPLC. The result showed that the methanolic extracts have a higher concentration of berberine when compared to other solvent fractions[101]. The high performance liquid chromatography (HPLC) method was validated for accuracy, precision, reproducibility and robustness for the estimation of cordifolioside A in 60% methanolic extract of *T. cardifolia*. Statistical analysis proved that the method is precise, reproducible, selective, and accurate for the estimation of cordifolioside A. HPLC method was validated for accuracy, precision, reproducibility, and robustness as per International Conference on Harmonisation (ICH) guidelines[102,103].

IMMUNO-MODULATORY ACTIVITY OF TINOSPORA CARDIFOLIA

T. cardifolia is well researched for its immuno-modulatory activities since many years and few compounds have been isolated and subjected for their possible mode of immuno-modulatory activity. According to a recent report, arabinogalactan polysaccharide (G1-4A) from the stem, appear to induce tolerance against endotoxic shock by modulation of cytokines and nitric oxide (NO). In addition, it also modulated the release of nitric oxide by marine macrophages[104].The immunostimulating signaling mechanism of the novel (1,4)-alpha-d-glucan reported to be through the activation of macrophages that occurred through TLR6 signaling, NF-kappa B translocation and cytokine production[105]. Polysaccharide fraction from *T. cardifolia* is found to be very effective in reducing the metastasis potential of B16F-10 melanoma cells[106]. Arabinogalactan polysaccharide has good protective effect against iron mediated lipid peroxidation of rat brain homogenate as revealed by the thiobarbituric acid reactive substances and lipid hydroperoxide assays[107]. The effect of aqueous extract of *T. cordifolia,* an immunomodulator with antimalarial activity along with chloroquine was studied in the treatment of three cases of hyper-reactive malarious splenomegaly in District Hospital, Daltonganj town, Jharkhand, India. Addition of extract of *T. cordifolia* for the first six weeks to chloroquine showed regression of spleen by 37–50% after six weeks and 45–69% after six months from the start of treatment[108].

ANTIHYPERGLYCAEMIC ACTION

T. cardifolia has been extensively studied for its hypoglycemic activity in support of its usefulness in the treatment of diabetes mellitus. An Ayurvedic polyherbal formulation 'Ilogen-Excel', which contains *T. cardifolia* as one of the constituent, administered at the dose of 50 mg/kg and 100 mg/kg for 60 days has shown a significant decrease in the blood glucose levels and increase in the plasma insulin, hepatic glycogen and total hemoglobin[100]. One more herbomineral formulation “Hyponidd” is reported for its possible hypoglycemic as well as antioxidant activity and the results are comparable with earlier reports on this plant[109]. Diabetics are prone to the development of cataract; alcohol extract of *T. cardifolia* has preventive effect on the development of cataract and produces a significant reduction of plasma glucose levels in alloxan induced diabetic rats[110]. The effects of *T. cardifolia* extracts on blood glucose concentrations as well as on kidney functions in streptozotocin induced diabetic rats have been investigated. The extract when administered orally for 40

days was found to decreased plasma glucose concentration and prevented polyuria, rise in urinary albumin levels and renal hypertrophy as well[111]. In India, the decoction of kernels of *Eugenia jambolana* and extracts of *T. cardifolia* are used as a household remedy for diabetes. The anti-hyperglycemic effect of aqueous and alcoholic extracts as well as lyophilized powder of these two plants was evaluated in diabetic animals[112]. The stem of *T. cardifolia* is widely used in the therapy of diabetes by regulating the blood glucose in traditional folk medicine of India and it has been reported to mediate its anti-diabetic potential through mitigating oxidative stress (OS), promoting insulin secretion and also by inhibiting gluconeogenesis and glycogenolysis, thereby regulating blood glucose[113]. The isoquinoline alkaloid rich fraction from stem, including, palmatine, jatrorrhizine, and magnoflorine have been reported for insulin-mimicking and insulin-releasing effect both *in vitro* and *in vivo*[114]. The protective effects of *T. cardifolia* were reported in the presence of higher levels of anti-oxidant molecules and enzymes[37]. *T. cardifolia* has been shown to significantly counterbalance the diabetes-associated OS in the maternal liver by lowering the levels of malondialdehyde and ROS and the increased levels of GSH and total thiols[115].

THERAPEUTIC APPLICATIONS

The ethanolic extract of the root of *T. cardifolia* was observed to induce a marked protective action against restrain stress induced ulcerization. The antiamoebic activity of crude drug formulation containing *T. cardifolia* against *Entamoeba histolytica* showed varying degrees of inhibition of the enzymes i.e DNase, RNase, Aldolase, alkaline phosphatase, acid phosphatase and protease activities of crude extracts of axenically cultuted amoeba. The water and ethanolic extracts of stem of the plant inhibited immunosuppression produced by cyclophosphamide. The ethanolic extract of stem of the plants inhibits cyclophosphamide induced anemia. Water extract was more potent than any other extract of the plant[116].

ANTIOXIDANT/HEPATOPROTECTIVE ACTIVITY

The antioxidant activity of an arabinogalactan polysaccharide i.e Tamarind seed polysaccharide (TSP) isolated from *T. cordifolia*, an Indian medicinal plant, was studied. The polysaccharide showed good protection against iron-mediated lipid peroxidation of rat brain homogenate as revealed by the thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LOOH) assays. TSP also provided significant protection to protein against γ-ray induced damage. The protective action can possibly be explained by its very high reactivity towards 2,2-diphenyl-1-picrylhydrazyl(DPPH), superoxide radicals and the most damaging of radicals i.e the hydroxyl radical[117]. The 50% alcoholic extracts of *Swertia chirayita*, *Cedrus deodar, Boerhavia diffusa*, *Berberis aristata*, *Withania somnifera*, *Pongamia glabra*, *Petrocarpus santalinus*, *T. cardifolia* and *Acrous calamus* were screened for antioxidant and possible anti-inflammatory potential. Among the tested plants, only *Pongamia glabra, Petrocarpus santalinus, T. cardifolia*, and *Acrous calamus* were found to possess hydroxyl radical scavenging activity[118].

CYTOTOXIC EFFECT

The anti tumor activity of *T. cardifolia* may be due to decreased lipid peroxidation, glutathione-S-transferase (GST) activity or due to the release of lactate dehydrogenase. Dichloromethane extracts showed concentration dependent decline in the clonogenicity, glutathione-S-transferase activity as well as increased in lipid peroxidation with a peak at 4 h and a lactate dehydrogenase release with a peak at 2 h[119]. Exposure of HeLa cells to 0, 5, 10, 25, 50 and 100 *μ*g/ml of guduchi extracts (methanol, aqueous and methylene chloride) resulted in a dose-dependent but significant increase in cell killing, when compared to non-drug-treated controls. The effect of guduchi extracts was comparable or better than doxorubicin treatment. The results demonstrate that guduchi killed the cells very effectively *in vitro* and deserves attention as an antineoplastic agent[38]. Administration of

T. cordifolia stem methanolic extract to mice (200 mg/kg, i.p, daily for 5 days) increased the total white blood cell count significantly (P<0.001). It also increased bone marrow cellularity (18.16×10^6/femur) and α-esterase positive cells (1423/4000 cells) in bone marrow indicating increased maturation of stem cells. *Tinospora* extract reduced solid tumor growth and synergistically acted with cyclophosphamide in reducing (83%) the animal tumors . *T. cardifolia* extracts have been shown to up regulate the anti tumor activity of macrophages (TAM). Evidence has shown that an alcoholic extract of *T. cordifolia* (ALTC) enhances the differentiation of TAM to dendritic cells (DC) in response to granulocyte/macrophage-colony-stimulating factor, interleukin-4, and tumour necrosis factor 7[120]. Cyclophosphamide (CP) an anti-cancer drug has been reported to reduce the GSH content in both bladder and liver and lowered levels of cytokines Inerferon-γ and IL-2 an increased levels of pro-inflammatory cytokine TNF-α. This effect could be reversed on *T. cardifolia* treatment indicating the role of *T. cardifolia* in overcoming CP induced toxicities in cancer treatment[121].

OTHER ACTIONS

The usefulness of *T. cardifolia* as a cognitive enhancer was substantiated by its potent *in vitro* acetylcholinesterase inhibitory activity. Metha mnolic and successive water extracts have been investigated, whereby, methanolic extract was found to be more active than water extract[102]. The efficacy of the extract in patients of allergic rhinitis was assessed in a randomized double blind placebo controlled trial which demonstrated a significant decrease in all the symptoms of allergic rhinitis[98]. Methanolic (70%) stem extract has significant male antifertility activity. In male rats, it has no effect on body weight loss but decreases the weight of testes, epididymis, seminal vesicle and ventral prostate in a significant manner. It reduces the sperm motility as well as sperm density significantly, which result in reduction of male fertility by 100%[53]. *T. cardifolia* can be exploited for the protection against gamma irradiation in mice. This property can be exploited to human applications for radioprotective manifestation. A preparation of *T. cardifolia* administered to mice at a dose of 200 mg/kg and an aqueous extract at 5 mg/kg for 1 hour before whole body gamma irradiation, exhibits a significant protective effect. The direct and indirect antioxidant actions of *T. cardifolia* acts in corroboration to manifest the overall radioprotective effects[100,122].

ANTI-ARTHRITIC AND ANTI-OSTEOPOROTIC EFFECTS

Single or synergistic formulations of *T. cordifolia* with *Zingiber officinale* has been used in rheumatoid arthritis treatment in traditional medicine[123]. *T. cordifolia* have been reported to affect the proliferation, differentiation and mineralization of bone like matrix on osteoblast model systems *in vitro* and hence finds potential application as an anti-osteoporotic agent. Alcoholic extract of *T. cordifolia* have been shown to stimulate the growth of osteoblasts, increasing the differentiation of cells into the osteoblastic lineage and also increasing the mineralization of bone like matrix. Beta-Ecdysone (Ecd) from *T. cardifolia* extracts have been reported to induce a significant increase in the thickness of joint cartilage, induce the osteogenic differentiation in mouse mesenchymal stem cells and to relieve osteoporosis in osteoporotic animal models[124].

ANTI-HIV EFFECTS

T. cardifolia has been shown to demonstrate a decrease in the recurrent resistance of HIV virus thus improving the therapeutic outcome[125]. Anti-HIV effects of *T. cardifolia* was revealed by the reduction in eosinophil count, stimulation of B lymphocytes, macrophages and polymorphonuclear leucocytes and hemoglobin percentage thus, revealing its promising role of application in management of the disease[126].

NUTRIENT-NUTRIENT INTERACTIONS

Bacopa monnieri (Brahmi) and *Evolvulus alsinoides* (Shankhapushpi) are two ayurvedic herbs used for the purpose of cognitive

enhancement. The combination of these two herbs along with *T. cardifolia* appears to be most effective remedy against amnesia which suggests synergism[127]. Ginger is a Spice that is also traditionally used as an ayurvedic medicine and it is used alongside *T. cordifolia* for the treatment of rheumatism[123,128].

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