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**Review on Antimicrobial, Antioxidant, Cytotoxic activity
and Phytochemical analysis of *Drynaria quercifolia* and
*Decalepsis hamiltonii***

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Abstract

Medicinal plants are the most important source of life saving drugs for the majority of world's population. Over centuries cultures around the world have learned how to use plants to fight illness and maintain health. The World Health Organization estimates that 80 percent of the total world population depend on traditional remedies such as herbs for their medicine. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients. The most popular drug analgesic, aspirin and some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources. In India, around 20,000 medicinal plants have been recorded, however traditional communities are using only 7,000 -7,500 plants for curing different diseases. In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from traditional healers. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Several plants are used in folk medicine. Among them ferns are also used in different traditional medicinal systems of India and they play an important role in folklore medicine. A systematic survey of medicinal use of fern has been scarcely undertaken. Considering the need for novel drugs for the emerging new drug resistant microbial strains, the following two plants were selected for further studies.

Drynaria quercifolia, a non-flowering group of plant is being used by the tribals against skin diseases. An epiphytic fern *Drynaria quercifolia*, commonly called Oak leak Fern, is used in medicinal system by different groups of people to treat various kinds of health problems including urinary tract infection. Tribals in kalakad, Mundanthurai Tiger Reserve India, utilizes its rhizome to cure rheumatism.

Decalepsis hamiltonii is a monogeneric climbing scrub native of the Deccan peninsula and forest areas of Western Ghats of India. This is an endemic and endangered medicinal plant and grow largely in moist as well as dry deciduous forest, scrub jungles of southern parts of Deccan peninsula. The roots are being used in Ayurveda, the ancient Indian system, to stimulate appetite, relieve flatulence and as a general tonic.

Keywords: Medicinal plants, *Drynaria quercifolia*, *Decalepsis hamiltonii*.

Introduction

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 preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Lucy & Edgar, 1999).

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the north east, but it is thoughtless as art as old as mankind (Mahesh & Sathesh, 2008).

Herbal Medicine is defined as a branch of science in which plant based formulations are used to alleviate diseases. It is also known as botanical medicine or phytomedicine. Lately phytotherapy has been introduced as more accurate synonym of herbal or botanical medicine. In the early twentieth century herbal medicine was prime healthcare system as antibiotics or analgesics were not as yet discovered. With the advent of allopathic system of medicine, herbal medicine gradually lost its popularity among people, which is based on the fast therapeutic actions of synthetic drugs (Singh 2007).

Drynaria quercifolia

Drynaria quercifolia (L.) J. Smith of the family Polypodiaceae of Pteridophyta is distributed widely in the evergreen forests of India. Whole plant is anthelmintic, expectorant, tonic and used in the treatment of chest and skin diseases. The fronds have astringent properties and are found to strengthen and promote the repair of sinews, muscles and bones (Nejad and Deokule, 2009; Nadkarni and Nadkarni, 1976; Shil and Choudary, 2009).

Drynaria is an important medicinal plant. It is, commonly known as basket ferns, are found in wet tropical environments, usually in rainforests. The rhizome and frond of this plant is used in the treatment of hectic fever, dyspepsia and cough. It is used as anti-helminthic and astringent (Reynolds, 1985).

Decalepis hamiltonii

Decalepis hamiltonii Wight & Arn. is a monotypic, glabrous, climbing shrub belonging to the family Periplocaceae (earlier under Asclepiadaceae). This is an endemic and endangered medicinal plant and grows largely in moist as well as dry deciduous forests, scrub jungles of southern parts of Deccan Peninsula and the Western Ghats of India (Gamble and Fischer, 1957).

D. hamiltonii is one of the four species in the genus *Decalepis*. It is endemic to peninsular India and is known by various names like "Maredu Kommulu, Nannari kommulu and Madina Kommulu" in Telugu, "Makali ber" in Kannada and "Magali kizhangu" in Tamil. The plant root is used in ayurvedic medicines and in pickles (Anburaja et al, 2012). The habit of the plant is a liana. It is a globally endangered species (Raju & Ramana 2009). Regeneration is severely affected since most of the plants are harvested prior to seed setting.

D. hamiltonii roots are used as a substitute for *Hemidesmus indicus* in Ayurvedic preparations because of the similar aromatic properties (Nayar et al., 1978). People procure and habitually carry the roots with them and chew the same whenever the digestion may seek relief. This root extract is taken orally to rejuvenate the body (Reddy et al., 2006). These roots are encouraged to use in the form of powder and infusion to treat wounds and bronchial asthma (Manivannan, 2010). Recently it has been reported that roots of *D. hamiltonii* possess diuretic property (Arutla et al., 2012). The root extract of *D. hamiltonii* has been shown to contain significant antidiabetic, antiatherosclerotic and hepatoprotective properties (Naveen and Khanum, 2010).

The fresh roots of *D. hamiltonii* are available during monsoon in Southern parts of India and are generally dried and preserved for various food and pharmaceutical applications (Wealth of India, 1959). Roots of *D. hamiltonii* have traditionally been used as demulcent, diaphoretic, diuretic and tonic. It is used for treatment of the loss of appetite, skin diseases, diarrhoea, nutritious disorders, blood purifier, epilepsy and central nervous system disorders (Chopra and Nair, 1956).

Phytochemical analysis

Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (Vilegs et al, 1997). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952).

Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents. Any part of the plant may contain active components (Nair and Chanda, 2004).

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses. After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction (Sasidharan et al., 2011).

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples (Sasidharan et al., 2011).

Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (Cosa et al., 2006).

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, Soxhlet extraction and others are commonly used (Rockville, 2002; JP XIII, 2001) for the plant samples extraction.

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

Steroids and phlobatannins were found to be present in all the plants. It has been found that some of these investigated plants contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001).

Lali and Sukirtha (2012) in the phytochemical analysis of the plant extracts showed the presence of alkaloids, flavonoids, saponins, steroids, glycosides, phenolics and tannins in pomegranate leaves.

Egwaikide and Gimba (2007) reported that the medicinal properties of the plant could be attributed to the presence of one or more of the detected plant natural products. Ethyl acetate extract of *Plectranthus glandulosus* contain flavonoids such as quercetin which has antioxidant properties.

Phytochemical Analysis of *Drynaria quercifolia*

Drynaria quercifolia (L.) J. Smith belongs to Pteridophyta, and family Polypodiaceae. The plant is an epiphytic fern with a short thick, fleshy, creeping rhizome (Kandhasamy et al, 2008). It is found to be growing in rain forest of Western Ghats of Maharashtra, India. Batool and Subhash (2009) first reported on the antifungal activity of the ethanolic extract of *D. quercifolia* rhizome on dermatophytic species.

The ethanolic extract of *D. quercifolia* rhizome did not show any inhibition in the concentration of up to 20mg ml⁻¹ by agar dilution method. However di-ethyl ether extract with semipolarity showed high affection against *T. mentagrophytes* that it was been inactive with ethanolic extract (inhibition zone of 25 mm diameter). Results on HPTLC indicated that the ethyl acetate extract of *D. quercifolia* rhizome contains coumarins (Wagner et al, 1984).

Mithraja et al (2012) reported the presence of phenols and tannin in all the six extracts of the rhizome of *Drynaria quercifolia* tested. Flavonoids showed positive result only in aqueous and ethanol extracts. The phytochemicals such as alkaloids, proteins, xanthoproteins, carboxylic acid and coumarins gave negative results.

Phytochemical Analysis of *Decalepis hamiltonii*

The root of *D. hamiltonii* has shown to contain a number of compounds like aldehydes, alcohols, ketones, sterols and triterpenes such as amyriin and lupeol derivatives (Murti and Seshadri, 1941a, b), resinol, saponins, tannins, inositol, fatty acids (Murthi and Seshadri, 1942). *D. hamiltonii* root has a strong aromatic odour and its volatile oil (0.68%) which contains 2-hydroxy-4-methoxy benzaldehyde (HMB, 96%) as a major compound. The presence of tannins, phlobatannins, saponins, flavonoids,

steroids, terpenoids, cardiac glycosides and reducing sugar in the crude extracts of *D.hamiltonii* roots was confirmed by Samyudurai and Thangapandian (2012).

The phytochemical analysis performed by Devi and Latha (2012) revealed the presence of flavanoids, saponins, tannins, steroids, cardiac glycosides. Early studies conducted by Murti and Sheshadiri (1941 c) also support the presence of aldehyde, inositols, saponins, amyryns and lupeols.

The results of phytochemical analysis of various solvent extracts of *D. hamiltonii* by Samyudurai and Thangapandian (2012) reveals the presence of tannins, alkaloids, glycosides, flavanoids and phenols. From this analysis, methanol extracts of stem bark and root were found to have more chemical constituents compared to other extracts. Giridhar et al (2005) have reported the presence of 2-hydroxy-4-methoxy benzaldehyde a flavor compound in plants derived in vitro.

According to Vedavathy (2004) the roots of *Decalepsis hamiltonii* are little bitter and then sweet. It is so characteristic with familiar taste and smell of vanillin the substance used in chocolates, drinks and ice creams. Though vanillin is synthesized artificially there is always a demand for natural compound. The roots of *Decalepsis* can be used as a substitute for vanillin.

Antimicrobial activity

Synthetic drugs produce many side effects to the users and therefore anti microbial drugs from plants have received importance (Tomlin and Tomasz, 1986). Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (Gordon & David, 2001).

There are several reports on the antimicrobial activity of different herbal extracts (Samy P R, Ignacimuthu S, 2001). Higher plants play an important role, by producing large number of organic compounds as secondary metabolites, which can be used as self-defense. They act as bioactive compounds, chemotherapeutic, bactericidal, and bacteriostatic agents (Evans et al., 1986; Purohit and Bohra 1998)

The antibacterial activity of different plant species was evaluated by agar disc diffusion method for aqueous extract and agar well diffusion for solvent extract using Mueller Hinton agar No. 2 medium for the assay (Nair R & Chanda S, 2007).

Antimicrobial activity of *Drynaria quercifolia*

Kandhasamy et al (2008) studied the efficacy of different extracts of *D. quercifolia* on antibacterial activity. Ethanolic and methanolic extracts of the rhizome of *D. quercifolia* exhibited broad spectrum of antibacterial activity. The ethanolic and methanolic extracts inhibited

the growth of pathogenic bacteria 80 and 70% respectively. It was observed that gram negative bacteria were more sensitive to most of the extracts tested compared to gram-positive bacteria.

Mithraja et al (2012) evaluated the antimicrobial efficacy of the rhizome extracts of *Drynaria quercifolia* against the clinically isolated human pathogens from infected urinary tract. The acetone and ethanol extracts from rhizome of *Drynaria quercifolia* shows antibacterial effect on clinically isolated bacterial pathogens of urinary tract such as *Streptococcus pyogenes*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Mohanta et al (2013) studied the antimicrobial activity of different rhizome fractions of *Drynaria quercifolia* and reported mild antimicrobial activity in ethyl acetate and carbon tetra chloride fractions.

In studies conducted by Batool and Subhash (2009) the solvents of acetone, methanol and water extracts of *Drynaria quercifolia* did not also show any efficacy by disk diffusion method. Di-ethyl ether extract with semipolarity showed high affection against *T. mentagrophytes* that it was been inactive with ethanolic extract.

Ramesh et al (2001) reported that methanolic extract of *D. quercifolia* rhizome showed inhibitory activity against bacteria by the agar-well diffusion method but showed negative activity against fungi. Methanolic extract of *D. quercifolia* rhizome showed inhibitory activity against all tested bacteria like *Klebsiella pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *S. aureus* and *Bacillus subtilis*. But no activity was observed against fungi tested like *Aspergillus flavus*, *A. niger* and *Candida albicans*.

Antimicrobial activity of *Decalepsis hamiltonii*

Phadke et al., (1994) investigated the essential oil of *D. hamiltonii* for its antimicrobial activity. The oil inhibited the growth of *Escherichia coli*, *Salmonella typhi* and *Saccharomyces*. He reported that the major antimicrobial component of essential oil responsible for the inhibition of microbial growth was HMB.

Thangadurai et al., (2004) studied the antimicrobial activity of different solvent petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts of *D. hamiltonii* roots. In this study it was found that among the 15 organisms tested growth of 11 pathogens were inhibited.

Elizabeth et al. (2005) also reported the strong antimicrobial property of *D.hamiltonii* root extracts. Studies by Kumuda et al., (2011) showed that petroleum ether and chloroform extracts had significant activity against *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella paratyphi B*.

Thangavel et al., (2011) investigated the antibacterial activity of crude petroleum ether extract obtained from leaf callus tissue of *D. hamiltonii* was against five bacterial pathogens. Maximum inhibitory activity was reported against *S.typhi*.

Mohana et al., (2008) also investigated the antimicrobial potential of *D. hamiltonii* against human pathogenic bacteria. The study was conducted in combination with other medicinal plants. They have also reported antifungal activity of *D. hamiltonii* against phytopathogenic fungi.

Devi and Latha (2012) reported the presence of flavanoids, saponins, tannins, steroids, cardiac glycosides in phytochemical analysis. The antibacterial activity was studied by disc diffusion method and all the extracts were found to possess different degrees of antibacterial activities except aqueous extract.

Samydurai and Thangapandian (2012) studied the antifungal activity of extracts taken of roots, stem bark and leaves of *Decalepsis hamiltonii* against *Candida albicans* using micro dilution assay. All the extracts exhibited moderate activity against *Candida albicans*. The results showed that the extracts from roots and stem bark had considerable activity than the leaf extract.

Antioxidant activity

Oxidation and reduction reactions are essential to many living organisms for the production of energy to biological purposes. However, oxygen free radicals and other reactive oxygen species (ROS) which are continuously produced in vivo, result in cell death and tissue damage. These species can react with biological substrates such as DNA and proteins, leading to several diseases including cancer, diabetes, cardiovascular diseases, aging, arthritis and atherogenesis (Halliwell and Gutteridge, 2007; Willcox, 2004)

Antioxidants are vital substances which provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal, 1996; Ozsoy, 2008). Several anti-inflammatory, antinecrotic, neuroprotective, chemopreventive and hepatoprotective drugs have recently been shown to have antioxidant and radical scavenging mechanism as part of their activity (Lin and Huang, 2000; Repetto and Llesuy, 2002).

Naima et al (2012) reported that analysis of free radical scavenging activity and total phenolic and flavonoid content showed that mainly the chloroform extract, ethyl acetate extract and methanol extract from the whole plant of *Torilis leptophylla* can be the potent source of natural antioxidants. The results of in vivo studies suggest that methanolic extract of *T. leptophylla* may be

useful in defense against CCl₄-induced liver damage possibly be due to its antioxidant properties.

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999). There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Sanchez-Moreno, 2002; Schwarz, et al., 2001).

Mohanta et al (2013) measured the antioxidant activity of different rhizome fractions of *Drynaria quercifolia* on the basis of its DPPH scavenging activity which showed strong antioxidant activity.

Sasikumar et al., (2014) evaluated the polyphenolic composition and antioxidant properties of methanol extract of rhizome of *Drynaria quercifolia* (L.) Sm by DPPH assay, hydroxyl ion radicals ($\cdot\text{OH}$), nitric oxide (NO), hydrogen peroxide (H_2O_2) and 2, 2'-Azinobis (3-ethylbenzothiazoline sulphonic acid) ABTS scavenging assays. The antioxidant capacities of the extract were stronger than that of the antioxidant standard, butyl hydroxy toluene (BHT) when compared with other medicinal ferns.

Lokesh et al (2014) measured the reducing power ability in the methanol and aqueous extracts of *Drynaria quercifolia*. showed higher activities than the control in dose dependent manner. The IC₅₀ value was 49.83 µg for MEDQ and 41.5 µg for AEDQ Whereas, IC₅₀ value of standard ascorbic acid was 12.39 µg. In DPPH Free radical scavenging activity the IC₅₀ values of MEDQ (53.60 µg) shows highest superoxide scavenging activity than AEDQ (66.20 µg) but their activity was less than standard ascorbic acid (4.30 µg).

Murthy et al (2006) studied the antimicrobial activity of extract from *Decalepsis hamiltonii* by different methods like DPPH assay, β -Carotene linoleate method, hydroxyl radical scavenging method. Results show strong antioxidant activity by the extracts and 2-hydroxy, 4-methoxy benzaldehyde is the major compound responsible for the antioxidant activity.

Srivastava et al., (2006) reported high antioxidant activity in methanolic extract and aqueous extracts of *Decalepsis hamiltonii* by DPPH assay. In 2007 Srivastava et al., Isolated a new anti oxidant compound ellagic acid from the aqueous extract of roots of *D. hamiltonii* and it was mainly responsible for antioxidant activity. Superior antioxidant activity of *Decalepis* rhizome of all the selected medicinal plants was reported by Naveen et al. (2011).

Cytotoxic activity

According to Samaha et al (1997) cytotoxicity is a complex event *in vivo* and could be direct cellular damage, physiological effects, systemic effects, inflammatory effects and other systemic effects. Cytotoxic agents unselectively kill and damage both normal and cancerous cells by interfering with either, the cellular process or mechanical process. The unspecific action of cytotoxic agents constitutes a major drawback in any therapy especially cancer chemotherapy. It is different with anticancer agents which are designated to kill the cancerous cells.

Currently, the non-radioactive, calorimetric assay system using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) has been widely used for evaluating cell viability *in vitro*. The assay measures the conversion of MTT into purple-colored MTT formazan by living cells and decrease in cellular MTT reduction could be an index of cell damage (Abe and Matsuki, 2000).

Mohanta et al (2013) reported significant cytotoxic potential in rhizome extracts of *Drynaria quercifolia* using Brine-shrimp lethality bioassay. Saetung et al studied the cytotoxic activity of Thai medicinal plant extracts. They reported that the ethanolic extracts of four plants showed cytotoxic activity against lung and prostate cancer cells.

Khan et al., (2011) assessed the cytotoxicity of extract of *Drynaria quercifolia* was assessed by brine shrimp lethality bioassay and LC50 values were determined. The LC50 values for petroleum, chloroform and ethyl acetate extracts were 22.0, 16.5 and 16.5 µg/ml, respectively.

Devi & Latha (2015) studied the *in vitro* cytotoxicity activity in methanolic extract of *Decalepis hamiltonii* root by MTT assay method. Two types of cell lines i.e. Vero cell line and A₅₄₉ cell line (cancerous cell line) were used for the study. The results showed significant anti-cancer activity. The antitumor activity of methanolic extract of *Decalepis hamiltonii* is probably due to its flavanoid content.

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