A review on anti-microbial and hepatoprotective properties of himalayan wild fern *Nephrolepis cordifolia* (Pani Amla)

Ujjal Chettri, Swati Kumari and Bikkey Chettri

Abstract

According to WHO not only microbial drug resistance but liver diseases is emerging as another burden for the people around the globe where about 1 million death occurs per year due to liver cirrhosis and other 1 million death due to viral hepatitis and hepatocellular carcinoma. In many studies plant based phytochemical component such as oleanolic acid, eugenol, β-ionone are found to be effective against several drug resistant microorganisms like *Streptococcus pneumoniae*, Methicillin resistant *Staphylococcus aureus* (MRSA) and *Vibrio parahaemolyticus* etc and this oleanolic acid and β-sitosterol derivatives are also found to be effective against various liver inflammation and helps in reducing the hepatocellular necrosis of liver. all this phytochemicals are found to be present in himalayan wild fern *Nephrolepis cordifolia*. In this review we have discussed about several phytochemical compounds which were naturally present in Himalayan wild fern *Nephrolepis cordifolia* (Pani Amla) and their antibacterial, antifungal, anticancer (anti tumor) and hepatoprotective properties.

Keywords: *Nephrolepis cordifolia*, antibacterial, antifungal, anti cancer, oleanolic acid, β-sitosterol

Introduction

*Nephrolepis cordifolia* is a terrestrial fern which grows vigorously by forming colony with short rhizome and small scaly tubers at their roots. They are able to grow in different situation: as epiphytic and epilithic plant with frond which are normally 16-32 inches long and 4 inches wide and appear as bright green color [1, 2]. It can spread with the help of spores, stolons, tuber and rhizomes and are commonly known as erect sword fern, lemon butter fern, ladder fern and fish bone fern [3]. In Tamil it is known as moothirakilangu, In Malaysia sarawak, and are commonly known as pani amla in hilly region of Sikkim [4]. This fern are edible and cooked as vegetables by the different tribes inhabiting the Himalayas. Since ancient time this fern were part of Indian traditional medicine and are used as diuretic, contraceptive and to treat liver disorders [5].

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**Fig 1:** *Nephrolepis cordifolia* plant (a) leaves growing on a rachis and (b) grown tubers of *Nephrolepis cordifolia*
The tubers of this plant contains high amount of moisture, fat, carbohydrate and calcium whereas the protein content was high in the part of rhizome. Fresh tubers of *Nephrolepis cordifolia* are roasted and consumed by the locals in Nepal [6]. *Nephrolepis cordifolia* plants are grown commonly as an ornamental fern in different areas of shillong [7].

**Morphological Features of Nephrolepis cordifolia**

**Frond or Pinnae**
Sterile frond are pinnate and grows up to 3 feet in height and 3 inch wider. each side of rachis contains numerous pinnae which are about 1.5-4 inches. Each pinnae growing on rachis are oblong or lanceolate in shape where each pinnae bearing numerous spore containing structure called sori, this sori or spore producing structure are produced between midvein of leaflets and margins [8] as shown in fig.2.

![Fig 2(A): back side of frond with pinnae containing spores (sori) (B) front side of frond with pinnae and brown coloured rachis](Image)

**Rhizomes and Tuber**
Rhizomes are densely clothed with brownish scales, with fleshy, egg-shaped tubers [9]. *Nephrolepis cordifolia* produces round fleshy tubers which are spherical about 15 mm arises from the network of creepy stems [10]. Tuber of *Nephrolepis cordifolia* contains high amount of moisture content this high level of moisture content present in food sample provides high activity for water soluble enzymes and co-enzymes useful for various metabolic activities [11].

**Phytochemical Constituents**
The different parts of *Nephrolepis cordifolia* like leaf, rhizome and rachis are covered very densely by epidermal glands. These epidermal glands contains various phytochemical substances like phenolic acids, flavonoids, glycosides and alkaloids. Due to presence of this substances they shows antimicrobial activity against several microorganism and they are found to be less soluble in water and highly soluble in organic solvents like ethanol, acetone and methanol [12-14]. According to the phytochemical screening of *Nephrolepis cordifolia* extract components like reducing sugar, tannins and cardiac glycosides are highly present [15]. In one of the experiment 6 different types of compounds were extracted with the help of *Nephrolepis cordifolia* ethanol extract. all of the six isolated and identified compound are β-sitosterol, fern-9(11)-ene, myristic acid, oleic acid, triacontanol, hentriacontanoic acid and they are isolated for the first time from this plant [16]. In one of the study on chemical composition of essential oil of *Nephrolepis cordifolia* it is found that Nonanal (10.6%), β-ionone (8.0%), eugenol (7.2%) and anethol (4.6%) were present in very high concentration [17]. all of this compound are mentioned in Table.1 and structure of all compound is shown in fig.2.

<table>
<thead>
<tr>
<th>Compound Type</th>
<th>Identified compound in <em>Nephrolepis cordifolia</em></th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosterols</td>
<td>β-sitosterol is present</td>
<td>LIANG Zhi-Yuan <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Fatty alcohol</td>
<td>oleanolic acid is present</td>
<td></td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>Fern-9(11) ene is present</td>
<td></td>
</tr>
<tr>
<td>Fatty acid</td>
<td>Myristic acid and Octadecylester is present</td>
<td></td>
</tr>
<tr>
<td>Aldehyde</td>
<td>Nonanal is present in essential oil.</td>
<td>Mona E. El-Tantawya <em>et al.</em> (2015)</td>
</tr>
<tr>
<td>Rose ketones</td>
<td>β-ionone is present in essential oil.</td>
<td></td>
</tr>
<tr>
<td>Phenylpropanoids</td>
<td>Eugenol is present in essential oil.</td>
<td></td>
</tr>
</tbody>
</table>
Therapeutic Properties of *Nephrolepis cordifolia* (Pani Amla)

Mostly free radicals are formed due to the oxidative metabolic process \[18\], production of this type of reactive oxygen species or free radicals leads to damage of cells and often leads to pathogenesis and acts as reason for other diseases like diabetes, cancer, alzheimers, and heart disease, liver disease etc.\[19\] action or oxidative stress produced due to this kind of free radicals can be reduced with the help of plant based phytochemicals. Plant based phytochemicals are becoming popular research of interest for researchers all around the world \[20\]. Plant extract of *Nephrolepis cordifolia* are found to contain phytochemicals like β-sitosterol, oleanolic acid, fern-9(11)ene, and its tuber extracted oil with presence of compound like nonanol, rose ketones, phenylpropanoid etc.\[21\] which shows different therapeutic properties like antibacterial, antifungal, anticancer and hepatoprotective activity.

**Antibacterial activity**

One of the study demonstrated that plant extract based oleanolic acid showed antimicrobial activity against vancomycin resistant enterococci and MIC of oleanolic acid against vancomycin resistant enterococci was 8 µg/ml. oleanolic acid also showed antimicrobial activity against *Streptococcus pneumoniae* and Methicillin resistant *Staphylococcus aureus* (MRSA) \[22\], a compound fern-9(11)-ene is found to be susceptible against gram negative bacteria except E. coli and highly susceptible against bacteria *Salmonella typhi* and moderately active against *Pseudomonas aeruginosa* \[23\]. One of the experiment studies on antibacterial and antibiofilm activity of eugenol against *Vibrio parahaemolyticus* demonstrated strong biofilm properties of eugenol against environmental and clinical isolates of multidrug resistant bacteria *Vibrio parahaemolyticus* and 0.1% concentration of eugenol is able to decrease the biofilms by 3 and 2.5 CFU/cm² against both of the clinical and environmental isolates of bacteria *Vibrio parahaemolyticus* \[24\] as shown in fig.3

**Antifungal activity**

A number of studies have demonstrated the antifungal activities of oleanolic acid against several pathogenic fungi. There are some of the reports on the oleanolic acid derivatives that have been designed and evaluated as a potential therapeutic agents in the control of microbial diseases. One of the researcher designed and synthesized the 25 oxime ester derivatives of oleanolic acid to study their anti-fungal activities and found that all of the oleanolic acid derivatives at concentration of 50 µg/ml showed a potential antifungal
activity against fungi like Sclerotinia sclerotiorum and Rhizoctonia solani [25]. In one of the study done on effect of eugenol on 31 strains of Candida albicans it is found that eugenol inhibits budding and mycelia formation completely and shows MIC of 625µg/ml for all of the tested strains of Candida albicans [26]. Other study on the β-Ionone molecule effect on P. expansum it is found that at 0.625% concentration of the β-Ionone is significantly able to inhibit the growth of P. expansum and with no any visible growth of its spores after incubation time [27].

Anti tumor/ Anti cancer activity
Different in vivo and in vitro studies reported anti tumor and anti cancer activities of oleanolic acid against tumor and growth of cancer. oleanolic acid is found to inhibit the transplanted tumour cell growth in mice and also able to inhibit proliferation of hepatocellular cells of liver. it is reported that antitumor activity of oleanolic acid depends upon tumor protein (p53) upregulation, mitochondrial apoptotic pathway activation mediated by cyclooxygenase-2 and cessation of the cell cycle progression [28]. In another study where cancerous cells of human bladder is treated with 50µM of oleanolic acid it is found that whole apoptotic pathway activation mediated by cyclooxygenase (COX), which consumes high concentration of UTP (uridine triphosphate) in order to inhibit the production of correlative enzymes and therefore prevention of necrosis of liver cells induced by CCl4, phalloidin acetaminophen et al, by reducing the level of serum transaminase leading to inflammation of liver [29].

Hepatoprotective properties
A pentacyclic terpenoid called oleanolic acid is a form of free acids and triterpenoid saponin glycosides present in plants [34-35] many of the animal studies demonstrated that oleanolic acid plays an important role in protection against injury of liver injury induced by CCl4, phallloidin acetaminophen etc, reducing the levels of serum transaminase leading to prevention of necrosis of liver cells [36-38] acute hepatic injury of liver is one of the life threatening syndrome which can be characterized by caugolopathy [39]. In one of the study done on effect of different factors like viruses, alcohols, and chemicals [40]. one of the hepatotoxic agent called Gal N (d-Galactosamine) which consumes high concentration of UTP (uridine triphosphate) in order to inhibit the production of correlative proteins of the liver leading to inflammation of liver and cause hepatic necrosis [41]. Lps (lipopolysaccharide) are responsible for stimulating immune cells which releases various inflammatory factors, leading to the apoptosis and necrosis of hepatic cells of liver [42-43]. In one of the study it is found that β-sitosterol derivative sitosterol-N when used in different concentration (12.5mg/kg) and (50mg/kg) helps in treatment and able to reduce the necrosis of liver cells induced by lipopolysaccharide and d-Galactosamine [44] as shown in fig.4.

![Histological changes of liver tissues](https://doi.org/10.1016/j.bmcl.2018.03.073)

Fig 4: Histological changes of liver tissues were observed under microscope at magnification of 400X. (A) as Control , (B) Induced with LPS and GalN, (C) Induced LPS /GalN + Sitosterol derivative (12.5 mg/kg) , (D) LPS/ Gal N + Sitosterol derivative (50 mg/kg). Central vein (black arrow), bleeding (red arrow), infiltrated neutrophils (yellow arrow), necrosis (blue arrow). (Source : https://doi.org/10.1016/j.bmcl.2018.03.073)
Conclusion
According to the WHO due to the inappropriate use of drugs many of the pathogenic microorganisms are becoming resistant and are emerging as a most challenging situation for researchers and also use of those synthetic drug are not really a good option due to its side effects to the body. Liver diseases is becoming another burden for the people around the globe where about 1 million death occurs per year due to liver cirrhosis and other 1 million death due to viral hepatitis and hepatocellular carcinoma. There are some components found in the *Nephrolepis cordifolia* like oleanolic acid, eugenol, β-ionone which shows antimicrobial properties against *Streptococcus pneumoniae*, Methicillin resistant *Staphylococcus aureus* (MRSA) and Vibrio parahaemolyticus etc. whereas the component like oleanolic acid and β-sitosterol derivatives are found to be effective against various liver inflammation and helps in reducing the hepatocellular necrosis of liver. Not only antibacterial and hepatoprotective properties but the components of *Nephrolepis cordifolia* are also effective against several pathogenic fungus and tumor or necrosis of hepatic cells. *Nephrolepis cordifolia* is one of the wild plant which are very less explored and which provides also effective against several pathogenic fungus and tumor or hepatocellular carcinoma. The *Nephrolepis cordifolia* grown in Egypt. Natural Product Research, 2015 http://dx.doi.org/10.1080/14786419.2015.1046070

References


8. https://plants.ifas.ufl.edu/plant-directory/nephrolepis-cordifolia/


17. Mona E El-Tantawyya, Manal M Shamsa, Manal S Afifi. Chemical composition and biological evaluation of the volatile constituents from the aerial parts of *Nephrolepis exaltata* (L.) and *Nephrolepis cordifolia* (L.) C. Presl grown in Egypt. Natural Product Research, 2015 http://dx.doi.org/10.1080/14786419.2015.1046070


