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Ujjal Chettri
Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

Swati Kumari
Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

Bikkey Chettri
Department of Botany, Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun, Uttarakhand, India

Corresponding Author:
Ujjal Chettri
Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

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].

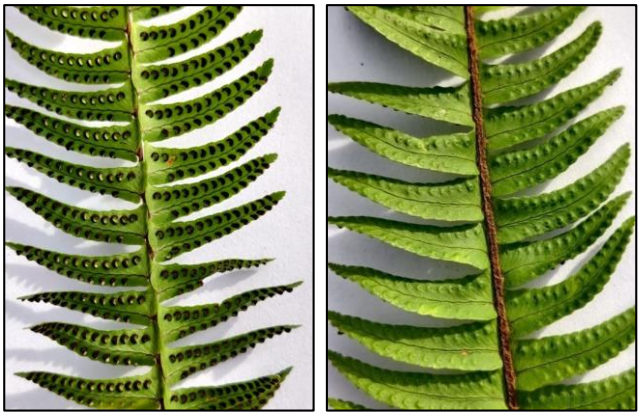
Fig 1: *Nephrolepis cordifolia* plant (a) leaves growing on a rachis and (b) grown tubers of *Nephrolepis cordifolia*

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 a ornamental fern in different areas of shillong [7].

Morphological Features of *Nephrolepis cordifolia*

Frond or Pinnae

Sterile frond are pinnate and grows upto 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 produing structure are produced between midvein of leaflets and margins [8] as shown in fig.2



A B

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

Rhizomes and Tubers

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]. Tubers 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].

A. B.

Fig 3: (A) grown tubers of *Nephrolepis cordifolia* (B) roots of *Nephrolepis cordifolia*

Phytochemical Constituents

The different parts of *Nephrolepis cordifolia* like leaf, rhizome and rachis are covered very desnsely 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, oleanolic acid, triacontanol ,hentriacontanoic acid and they are islolated 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%), b-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 1: Compound identified from *Nephrolepis cordifolia* plant extract

Compound Type	**Identified compound in** (*Nephrolepis cordifolia*)	**References**
Phytosterols	β-sitosterol is present	LIANG Zhi-Yuan1 *et al*. (2008)
Fatty alcohol	oleanolic acid is present	
Triterpenoid	Fern-9(11) ene is present	
Fatty acid	Myristic acid and Octadecylester is present	
Aldehyde	Nonanol is present in essential oil.	Mona E. El-Tantawya *et al.* (2015)
Rose ketones	β-ionone is present in essential oil.	
Phenylpropanoid	Eugenol is present in essential oil.	

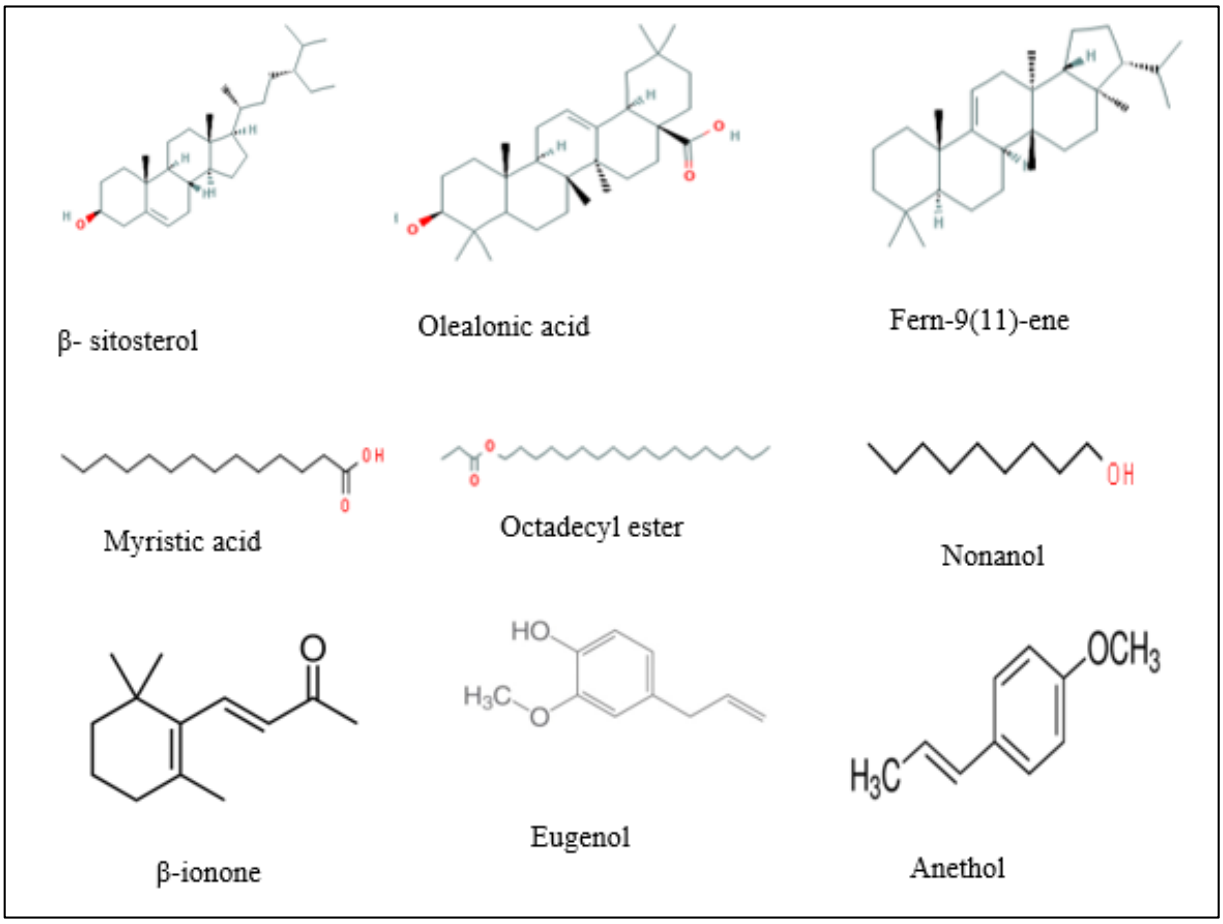


Fig 2: Structure of compound identified from *Nephrolepis cordifolia* extract (Source: Pubchem)

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, alzhmeirs, 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 enetrococci 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 Pseudomona aeruginosa [23]. one of the experiment studies on antibacterial and antibiofilm activity of eugenol against Vibrio parahaemolyticus demonstrated strong antibiofilm 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/cm^2 against both of the clinical and environmental isolates of bacteria Vibrio parahaemolyticus [24] as shown in fig.3

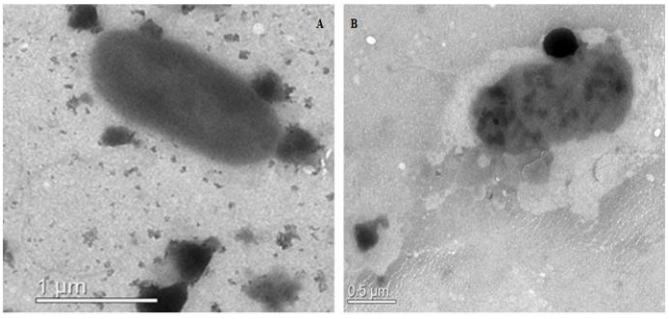


Fig 3: TEM images of Vibrio parahaemolyticus ATCC27969 (A) control cells of ATCC27969 (B) eugenol-treated cells of ATCC27969 (source: https://doi.org/10.1016/j.fm.2020.103500)

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 inhibibit 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 tomour 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 proliferation process stops and shutdown of apoptosis or program cell death occurs due to inhibition of different pathways.

Akt/mTOR/S6K and ERK1/2 responsible for signaling of cell growth, proliferation, and survival [29] one of the derivative of oleanolic acid which is oleanolic acid methyl estser with the help of induced apoptosis and production of reactive oxygen species in a time dependent manner are found to demonstrate cytoxic effect on cells of human cervical cancer (HeLa). All of this studies suggest that the oleanolic acid along with its derivatives can be valuable therapeutic agents against various tumor and cancerous cells and their mechanism [30]. In one of the study done by researchers on malignant melanoma cells it is found that eugenol acts as potent antiproliferative agent against malignant melanoma cells when treated with it [31]. recent a invivo study done on mice shows that immune response of organism is activated when β-sitosterols are administered to mice daily this study also shows high activity of cytokines IL-12 and IL-18 and natural killer cells decreasing high number of metastases in lung cancer cell line [32]. absorption of β-sitosterol at low degree shows slowing down of cell proliferation in colonic epithelial cells which further decrease the expression of neoplastic cells during its transformation [33].

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, phalloidin acetaminophen etc, by reducing the level 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] it may be caused due to 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 rna and protein 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

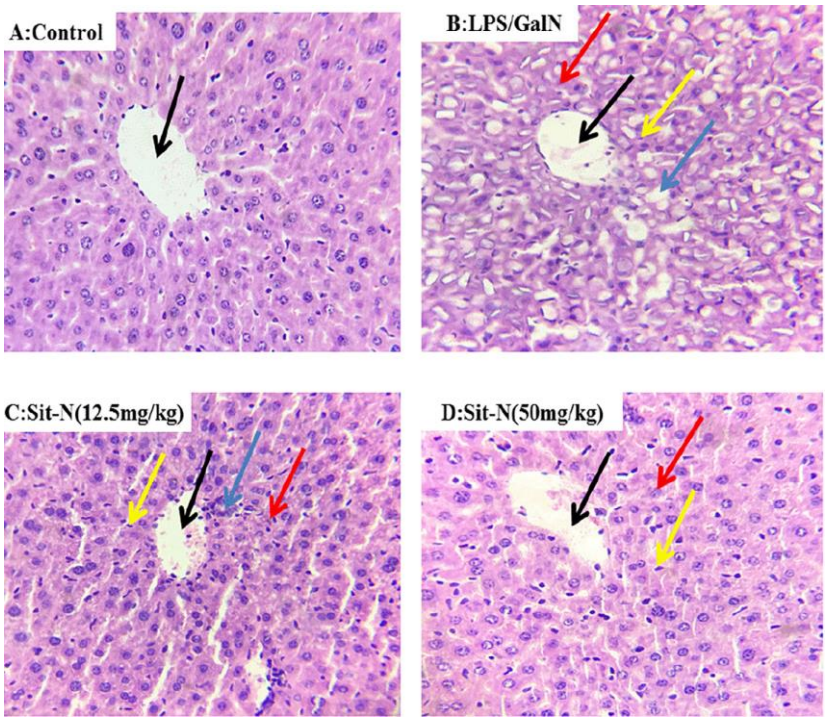


Fig 4: Histological changes of liver tissues were observed under microscope at magnification of 400X. (A) as Control , (B) Induced with LPS and GalN ,(C) Induced LPS /GalN + Sitosterol derivative (12.5 mg/kg) , (D) LPS/ Gal N + Sitosterol derivative (50 mg/kg) . Central vein (black arrow),bleeding (red 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 nercrosis of hepatic cells. *Nephrolepis cordifolia* is one of the wild plant which are very less explored and which provides opportunities for researcher in finding new phytochemicals hidden in it and it requires more research effort in determining its phytochemical composition. All of this naturally available compound in *Nephrolepis cordifolia* can be diversified into useful pharmaceutical product against multi drug resistant microorganisms and hepatocellular necrosis of liver.

References

1. Cheng L, Pu BJ, Zhou GY. The effects of physical and chemical factors on differentiation and growth of green globular bodies of *Nephrolepis cordifolia*. Journal of Tropical and Subtropical Botany, 2001; 9:142-148.
2. Segarra Moragues JG. Data about the subspontaneous Iberian pterydophyte flora: *Cyrtomium falcatum* (Dryopteridaceae), and *Nephrolepis cordifolia* (Nephrolepidaceae). Acta Botanica Malacitana. 2001; 26:247-249.
3. Schenk PM, Remans T, Sagi L, Elliott AR, Dietzgen RG, Swennen R *et al*. Promoters for pregenomic RNA of banana streak badnavirus are active for transgene expression in monocot and dicot plants. Plant Molecular Biology. 2001; 47:399- 412.
4. Tamang Mahendra, Pal Krishan, Santosh Kumar Rai. Traditional Use of wild plants for food in West Sikkim, India. Int. J of Life Sciences. 2017; 5(4):730-741.
5. Dhiman AK. Ethno medicinal uses of some pteridophytic species in India. Indian Fern Journal. 1998; 15(1, 2):61-64.
6. Gauchan DP, Dina Manandhar, Nisha Shrestha, Shyam Krishna Suwal. Nutrient Analysis of *Nephrolepis cordifolia* (L.) C. Presl. Kathmandu University Journal of Science, Engineering and Technology. 2008; I(V):68-72.
7. Chhetri RB. Trend in Ethnodomestication of some wild plants in Meghalaya, North East India. Indian J of Traditional Knowledge. 2006; 53(3):342- 347.
8. https://plants.ifas.ufl.edu/plant-directory/nephrolepis-cordifolia/
9. https://keyserver.lucidcentral.org/weeds/data/media/Html/nephrolepis_cordifolia.htm
10. http://www.stuartxchange.org/Bayabang.html
11. Rani D, Khare PB, Dantu PK. *In vitro* antibacterial and antifungal properties of aqueous and non-aqueous frond extracts of *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia*. Indian J Pharmaceut. Sci. 2010; 72:818-822.
12. Manickam VS, Benniamin A, Irudayaraj V. Antibacterial activity of leaf glands of *Christells parasitica* (L), Lev, Indian Fern J. 22:87-88
13. Maruzzella JC. Antimicrobial substances from ferns. Nature. 2005; 191:518-519.
14. Mickell LG. Antimicrobial activity of vascular plants. New York, Ecol. Bo1959t. 2005; 13:281-318.
15. Oloyede FA, Ajayi OS, Bolaji IO, Famudehin TT. An Assessment of Biochemical, Phytochemical and Antinutritional Compositions of a Tropical Fern: *Nephrolepis cordifolia* L. Ife Journal of Science. 2013; 15(3)
16. Liang Zhi-Yuan, Yang Xiao-Sheng, Zhu Hai-Yan, Hao Xiao-Jiang. Chemical constituents of *Nephrolepis cordifolia*. China National Knowledge Infrastructure. Guihaia, 2008, 3
17. Mona E El-Tantawya, 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
18. Singh R, Parihar P, Singh S, Mishra RK, Singh VP, Prasad SM. Reactive oxygen species signaling and stomatal movement: current updates and future perspectives, Redox Biology. 2017; 11:213-218.
19. Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P *et al*, Antioxidants of edible mushrooms, Molecules. 2015; 20:19489-19525.
20. Vilioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, Journal of Agriculture and Food Chemistry. 1998; 46:4113-4117.
21. Nugraha AS, Triatmoko B, Wangchuk P, Keller PA. Vascular Epiphytic Medicinal Plants as Sources of Therapeutic Agents: Their Ethnopharmacological Uses, Chemical Composition, and Biological Activities. Biomolecules. 2020; 10(2). DOI: 10.3390/biom10020181.
22. Kumiko Horiuchi, Sumiko Shiota, Tsutomu Hatano, Takashi Yoshida, Teruo Kuroda, Tomofusa Tsuchiya. Antimicrobial Activity of Oleanolic Acid from *Salvia officinalis* and Related Compounds on Vancomycin-Resistant Enterococci (VRE), Biological and Pharmaceutical Bulletin. 2007; 30(6):1147-1149.
23. Reddy VL, Ravikanth V, Rao TP, Diwan PV, Venkateswarlu Y. A new triterpenoid from the fern *Adiantum lunulatum* and evaluation of antibacterial activity. Phytochemistry. 2001; 56(2):173-175. doi:10.1016/s0031-9422(00)00334-4
24. Md Ashrafudoulla, Md Furkanur Rahaman Mizan, Angela Jie-won Ha, Si Hong Park, Sang-Do Ha. Antibacterial and antibiofilm mechanism of eugenol against antibiotic resistance *Vibrio parahaemolyticus*, Food Microbiology, Volume 91,2020,103500,ISSN 0740-0020,https://doi. org/10.1016/j.fm.2020.103500.
25. Taiwo Betty Ayeleso, Mashudu Given Matumba, Emmanuel Mukwevho. Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases Molecules. 2017; 22:1915; doi:10.3390/ molecules22111915

26. Boonchird C, Flegel TW. *In vitro* antifungal activity of eugenol and vanillin against *Candida albicans* and *Cryptococcus neoformans*. Canadian Journal of Microbiology, 1982; 28(11):1235-1241. https://doi.org/10.1139/m82-184
27. Barkai Hassan, Elabed Soumya, Guissi Sanae, Ibn Souda Koraichi Saad. Evaluation of the Antifungal Activities of Three Essential Oil Components against *Penicillium expansum* Spores. International Journal of Pharmacy and Pharmaceutical Sciences. 2017; 9(8).
28. Wang X, Bai H, Zhang X, Liu J, Cao P, Liao N, *et al*. Inhibitory effect of oleanolic acid on hepatocellular carcinoma via ERK–p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis. Carcinogenesis 2013; 34:1323-1330.
29. Mu DW, Guo HQ, Zhou GB, Li JY, Su B. Oleanolic acid suppresses the proliferation of human bladder cancer by Akt/mTOR/S6K and ERK1/2 signaling. Int. J Clin. Exp. Pathol. 2015; 8:13864
30. Song X, Liu CC, Hong YR, Zhu XC. Anticancer activity of novel oleanolic acid methyl ester derivative in HeLa cervical cancer cells is mediated through apoptosis induction and reactive oxygen species production. Bangladesh J Pharmacol. 2015; 10:896-902.
31. Ghosh R, Nadiminty N, Fitzpatrick JE, Alworth WL, Slaga TJ, Kumar AP. Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. J Biol. Chem. 2005; 280:5812-5819.
32. Awad AB, Fink CS, Williams H, Kim U. *In vitro* and *in vivo* (SCID mice) effects of phytosterols on the growth and dissemination of human prostate cancer PC-3 cells. Eur J Cancer Prev. 2001; 10:507-513.
33. Baskar AA, Al Numair KS, Gabriel Paulraj M, Alsaif MA, Muamar MA *et al*. β-sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer. J Med Food. 2012; 15:335-343.
34. Sultana N, Ata A. Oleanolic acid and related derivatives as medicinally important compounds. Journal of Enzyme Inhibition and Medicinal Chemistry. 2008; 23:739-756.
35. Pollier J, Goossens A. Oleanolicacid. Phytochemistry. 2012; 77:10-15. doi:10.1016/j.phyto chem.2011.12.022
36. Lu YF, Liu J, Wu KC, Klaassen CD. Protection against phalloidin-induced liver injury by oleanolic acid involves Nrf2 activation and suppression of Oatp1b2. Toxicology Letters. 2015; 232:326-332.
37. Yu Z, Sun W, Peng W, Yu R, Li G, Jiang T. Pharmacokinetics *in vitro* and *in vivo* of Two Novel Prodrugs of Oleanolic Acid in Rats and Its Hepatoprotective Effects against Liver Injury Induced by CCl4. Molecular Pharmaceutics. 2016; 13(5):1699-1710. doi: 10.1021/acs.molpharmaceut.6b00129.
38. Liu J, Liu Y, Madhu C, Klaassen CD. Protective effects of oleanolic acid on acetaminophen-induced hepatotoxicity in mice. Journal of Pharmacology and Experimental Therapeutics. 1993; 266:1607-1613.
39. Sogaard KK *et al*. Risk of venous thromboembolism in patients with liver disease: A nation wide population-based case-control study. Am J Gastroenterol. 2009; 104:96-101.
40. Andrade RJ *et al*. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005; 129(2):512-521.
41. Sathivel A *et al*. Sulfated polysaccharide isolated from *Ulva lactuca* attenuates d –galacto samine induced DNA fragmentation and necrosis during liver damage In rats. Pharm Biol, 2013.
42. Tsutsui H, Nishiguchi S. Importance of Kupffer cells in the development of acute liver injuries in mice. Int J Mol Sci. 2014; 15:7711-7730.
43. Decendit A *et al*. Malvidin-3-O-beta glucoside, major grape anthocyanin, inhibits human macrophage-derived inflammatory mediators and decreases Clinical scores in arthritic rats. Biochem Pharmacol. 2013; 86:1461-1467.
44. Yongxia Yin, Xiaofeng Liu, Jinping Liu, Enbo Cai, Hongyan Zhu, Haijun Li *et al*. Beta-sitosterol and its derivatives repress lipopolysaccharide/D-galacto samine - induced acute hepatic injury by inhibiting the oxidation and inflammation in mice, Bioorganic & Medicinal Chemistry Letters. 2018; 28:1525-1533.