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CLINICAL APPRAISALS AND PHYTOCHEMICAL POTENTIAL OF ETHNOMEDICINAL PTERIDOPHYTE: *DRYNARIA QUERCIFOLIA* (L.) J. SMITH (POLYPODIACEAE)

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Abstract

Drynaria quercifolia is a medicinal pteridophyte which have been treated by the administration of plant parts based on traditional and folk uses since ancient times. Considerable utilization and progress have been achieved regarding its biological activities. Various phytoconstituents like 3,4-dihydroxybenzoic acid, friedelin, epifriedelinol, coumarins β-amyrin, β-sitosterol and β-sitosterol 3-β-D-glucopyranoside has been isolated from the plant and these bioactive compounds responsible for its antidermatophytic, antimicrobial, antifertility, anti-lipidperoxidative, antiulcer, antipyretic, anti-arthritis, anti-urolithiatic, Pesticidal and Pest Repellency, thrombolytic and various other activities. Considering the ethnomedicinal significance of this parasitic fern, an attempt has been made for the first time to review to provide up to date clinical reports on the plant and to document the available phytochemical constituents through data base searches (PubMed, Google scholar, Scopus).

Keywords: pteridophyte, Drynaria quercifolia, phytoconstituents, dynaria, ethnopharmacology

Introduction

Plants used in traditional healthcare have become one of the main sources of drug discovery and development. From ancient times in China, women with low back pain have been treated with traditional Chinese medicines [1]. *Drynaria quercifolia* (L.) J. Smith (Polypodiaceae), commonly known as "Oak Leaf Fern", is found in Bangladesh, India, Pakistan, North America and Africa [2]. Traditionally, the soup prepared from the rhizome of *D. quercifolia* is very popular among tribes of Eastern Ghats, Tamil Nadu and the soup drink to get relief from rheumatic complaints [3]. The fern *Drynaria quercifolia* rhizome is used by local tribes in the rain forests of Western Ghats of Maharashtra, India. The rhizome is ground into a paste and used to treat diarrhea, typhoid, cholera, chronic jaundice, fever, headache, and skin disease [4]. In Bangladesh, *D. quercifolia* and its several plant parts has been used by the local inhabitants, folk and tribal medicinal practitioners to treat jaundice, hepatitis, Chest pain, diabetes, gonorrhea, debility and malaria [5-10]. In Lakshmipur district of Bangladesh, the rhizome of this plant is used by local people in the treatment of mental disorder [11]. Rhizome-paste with coconut oil, applied on head for the treatment of long sleeping disorder by the local people of Phulpur in Mymensingh and also used in the treatment of insanity by the local people of Netrakona [12]. In Southeast Asia rhizome decoction of *Drynaria quercifolia* uses as antipyretic preparation [13] and is also used topically in traditional Chinese medicine to stimulate hair growth and to treat baldness [14]. Oil obtained from the species is used as an in indigenous medicine, and the species is can be used as an ornamental plant [15]. In Malaysia fronds are used as poultice on swellings [16]. The results of the atomic absorption spectrophotometry showed that the level of lead, cadmium and copper were below the detection limits in *D. quercifolia* rhizome [17]. The full taxonomy of the plant is shown below in table 1. The ethnic people of Tripuri and Reang communities of Tripura, India are involved in using *D. quercifolia* leaves and rhizome for the treatment of intestinal worms and abdominal pain [18]. Peeled rhizome with sugar is prescribed by the marma tribes of Bangladesh for urinary disorders and in spermatorrhoea [19]. Tribals in Kalakad Mundanthurai Tiger Reserve, India, used the rhizome of this fern to cure rheumatism [20].

In Vietnam, the plant rhizome is used for the treatment of tuberculosis [21] rheumatism, osteodynia and dentagia [22].

Phytochemical Screening and Molecular Specification

The preliminary phytochemical investigation of the leaves of *D. quercifolia* showed (Table 2) the presence of phytochemical constituents such as alkaloid, glycosides, tannin, saponins, proteins and aminoacids, flavonoids, triterpenes, phenols, phytosterols and carbohydrate but absence of fats & fixed oils and gum and mucilages in different extractives [23]. Friedelin (yield: 0.15% on dried weight), epifriedelinol (0.1%), β-amyrin (0.09%), β-sitosterol (0.18%) isolated from hexane and CHCl3 combined extracts of *Drynaria quercifolia* [13]. β-sitosterol 3-β-D-glucopyranoside (0.24%) and naringin (0.09%) were isolated from MeOH extract. TLC studies revealed the presence of β-amyrin, β-sitosterol and catechin. Total phenolic compound of *D. quercifolia* was determined as 244 mg/g. The presence of the flavanone glycoside, naringin in *D. quercifolia* was established by HPLC and quantified as 0.048% [24]. Powdered rhizome of the plant five extracts were subjected to qualitative chemical evaluation, done [25] to detect the chemical constituents present in them. Petroleum ether extract revealed the presence of phytosterols, fixed oils and fats. The chloroform extract shows the presence of sterols.

The methanolic extract shows the presence of alkaloids, carbohydrates, glycosides, tannins, proteins and amino acids and the water extract has shown the presence of saponins, tannins, carbohydrates, proteins and amino acids (Table 3). A new natural product, namely propinqualin, whose structure was established as (-)-epiafzelechin-3-o-beta-D-allopyranoside isolated from rhizomes of Drynaria propinqua. 4-o-beta-D-glucopyranoside and sucrose were also isolated from *Drynaria propinqua* [26]. 3,4-dihydroxybenzoic acid and acetyl lupeol were isolated [27] from the rhizome of *Drynaria quercifolia* through bioassay-guided investigations.

Clinical Activities of *Drynaria quercifolia*

A great number of ethnopharmacological evaluations of *Drynaria quercifolia* has been reported till date. These are described in below:

Antimicrobial Activity

Anti-microbial substances derived from plants have received considerable attention in recent years [28]. *In vitro* the ethanolic extract of *D. quercifolia* rhizome was active against *A. flavus, A. terrus* and *Alternaria sp.* while it was inactive against *A. niger, C. glarata, C. albicans* and *C. tropicalis* [29]. The rhizome of the plant contains various bioactive compounds with

high degree of antimicrobial activity against various pathogens, including becteria pathogens of Urinay Tract Infections. Antibacterial study was carried out [30] on clinically isolated Urinary Tract Infecting (UTI) bacteria by disc diffusion method. Among the six extracts tested against eight different UTI bacteria, acetone extract was effective against *Enterococcus faecalis* and *Streptococcus pyogenes*, while ethanol extract was effective against *Pseudomonas aeruginosa*. *Streptococcus pyogenes* is a major human pathogen, causing diseases ranging from mild superficial infections of the skin and pharyngeal mucosal membrane, up to severe systemic and invasive diseases and autoimmune sequelae [31]. Friedelin, epifriedelinol, β-amyrin, β-sitosterol, β-sitosterol 3-β-d-glucopyranoside and naringin isolated from the methanol extract of dried rhizome from *Drynaria quercifolia* showed concentration-dependent broad spectrum of anti-bacterial activity [13] Significant zone of inhibition was recorded at a dose concentration of 50 mg/ml against *Chromobacterium violaceum, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Vibrio cholera, Vibrio parahaemolyticus, Bacillus subtilis, Staphylococcus aureus* and *Aeromonas hydrophil*.

Preliminary studies were conducted [32] on three plants including *Drynaria quercifolia* to determine activity against *Neisseria gonorrhoeae*. The extracts of *D. quercifolia* caused inhibition of *Neisseria gonorrhoeae* clinical isolates and World Health Organization (WHO) strains, more so than the multidrug resistant *Neisseria gonorrhoeae*.

Another study [33] have confirmed that the ethanolic and methanolic extracts of the rhizome of *Drynaria quercifolia* showed wide range of antibacterial activity. They have found nil activity in all the ten tested bacteria with Petroleum ether extract and Hexane extract. Benzene and chloroform extracts have shown mild activity. Irudayaraj and Senthamarai [34] have observed high degree of antimicrobial activity in ethanol extract of the rhizome against *Candida albicans, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa* with the inhibition zone range from 12-29 mm. They have reported the presence of steroid, phenolic groups, catechin and tannin with the very good positive result for catechin. 3.4-dihydroxybenzoic acid was isolated [27] from the rhizome of *Drynaria quercifolia* which showed significant antibacterial activity against four gram-positive and six gram-negative bacteria. The MIC values of 3.4-dihydroxybenzoic acid against tested gram-positive and gram-negative bacteria ranged from 8-32 and 16- 64 µg/ml, respectively These MIC values indicate the potency of the isolated compound against gram-positive bacteria is higher than that of gram-negative bacteria. Clinical studies have confirmed the beneficial effects of beta-sitosterol in patients with prostate enlargement. The phytochemical decreases post-void residual urinary volume and increases urinary flow rate in these patients [35].

The antibacterial potentials of *Drynaria quercifolia* rhizome of 125,250,500 mg methanolic extracts were screened against four human pathogenic bacteria using agar well diffusion method. The maximum zone of inhibition was observed in 500 mg of *Drynaria quercifolia* L. rhizome extract against *Escherichia coli* (12 mm) followed by *Bacillus subtilis* (10.3 mm), *Staphylococcus aureus* (10.3 mm) and *Salmonella sp*. (7.3 mm) which were higher than that of standard antibiotic streptomycin. Zone of inhibition of streptomicin were 10.1 mm, 10 mm, 8.3 mm and 7.3 mm for *Staphylococcus aureus*, *E.Coli, Salmonella sp*. and *Bacillus subtilis* respectively. The moderate results were observed in 250 mg of *Drynaria quercifolia* L. rhizome extract against *E. coli* (10.3 mm), *Staphylococcus aureus* (9.3 mm) and *Bacillus subtilis* (8.5mm). No inhibition was observed against *Salmonella sp*. Similarly least result were observed in 125mg of plant extract against *Staphylococcus aureus* (9.5mm) followed by *E. coli* (8.3mm) and *Bacillus subtilis* (8mm). No inhibition was observed against *Salmonella sp* [36].

Anthelmintic and Antifungal Activity

Anthelmintic activity of *D. quercifolia* was evaluated [37] using adult earthworms and piperazine citrate was used as a standard. Various doses (2.5, 5, 10, 25, 50 mg/ml) of alcoholic extracts of leaves and rhizomes of *D. quercifolia* were used. At all the tested doses, both extracts caused paralysis and also death of the worms. Though time taken for each concentration to paralyse and kill the parasite is comparatively longer than that for piperazine citrate, the results are highly significant ($P<0.01$). The activity also confirms dose dependent nature of the extract.

In Ayurvedic system of medicine, this epiphytic fern is called 'Ashwakatri' and it is used as pectoral, expectorant and anthelmintic agent [38]. The anthelmintic activity of *D. quercifolia* leaves extract was carried out [39] on earthworms. The extract exhibited dose dependent anthelmintic activity that causes paralysis at 6-23.67 min while death at 53.67-127.67 min. Albendazole (15 mg/ml) was used as reference standard (paralysis time at 35.33 min. and

death time at 71.33 min. Antifungal activity of methanol extracts *of Drynaria quercifolia* rhizome (125,250 & 500mg) were also assayed by agar well diffusion method against two pathogenic fungi. The maximum zone of inhibition was observed in 500mg of *D quercifolia* L. rhizome extract against *Trichophyton rubrum* (12.3 mm) which were higher than that of antibiotic action (8.6mm) and *Microsporum gypsum* (10mm) which were similar to that of standard antibiotic. 250mg extract showed the inhibitory activity against *Trichophyton rubrum* (8.3mm) and *Microsporum gypseum* (8.3mm) and least activity were found on 125mg of plant extract against *Trichophyton rubrum* (6.1mm) and *Microsporum gypseum* (7.6mm), [36].

Antioxidant Activity

Antioxidants are those substances capable of scavenging free radicals. All the extracts (Chloroform, methanol, aqueous) at a concentration of 500ppm have shown very good antioxidant activity. Among the rhizome extracts of *D. quercifolia* only methanolic extract at 500ppm has shown activity above 90%. Higher activity has been shown by the methanolic extract than standard α-tocopherol [40]. According to Lai and Lim [41], Methanol extract of *D. quercifolia* showed very high total phenolic content and is potent primary antioxidant as shown by its high radical scavenging capacity, reducing activity and BCB (β–carotene bleaching) antioxidant activity.

The anti-oxidant activity of different fractions of *D. quercifolia* was measured by the DPPH free radical scavenging activity. The concentration of petroleum ether soluble fraction, carbon-tetrachloride fraction, ethyl acetate soluble fractions and aqueous soluble fraction needed for 50% scavenging (IC50) of DPPH was found to be 161.68 µg/ml, 62.98 µg/ml, 38.25 µg/ml, 124.39 µg/ml, respectively. The positive control used as Butyl hydroxyl toluene (BHT) and for which the IC50 values were found to be 35.52 µg/ml [42]. Another study revealed that the rhizome methanol extracts of *D. quercifolia* also exhibited anti-oxidant property against DPPH, super oxide radicals and reducing power activity [43]. Recently, evaluation of *in-vitro* antioxidant study of *D. quercifolia* [44], concluded that methanolic extract of the plant could be one of the potential source of natural antioxidant for the treatment of free radical and age related diseases. The extract showed higher antioxidant activity in comparison to the ethanolic and hot water extract.

Antidermatophytic Activity

Results on high performance thin layer chromatography studies confirmed that the ethyl acetate extract of *D. quercifolia* rhizome contains coumarins and triterpenes [45]. The ethanol extract of the dried rhizome of *D. quercifolia* did not show inhibitory activity up to concentration of 20mg ml-1. The solvents of acetone, methanol and water also did not show any efficacy for extraction from *D. quercifolia* rhizome but di-ethyl ether with semi-polarity gave clear zone to antifungal activity compounds. Since coumarins soluble in semi-polar di-ethyl ether solvent, may be this compound can be responsible for antidermatophytic activity of this plant [46].

Antifertility Activity

The rhizome of the plant is reported to be used by different ethnic groups of India as a natural source of anti-fertility agent [47-48]. Some plant extracts can cause endometrial alterations resulting in nonreceptive endometrium and thus cause implantation failure [49-51].

The study [52] has demonstrated that the Methanolic extract of *D. quercifolia* rhizome possesses significant abortifacient and anti-implantation activity which may be attributed to the phytoconstituents of the plant. The mechanism of abortifacient and anti-implantation activities of *D. quercifolia* rhizome extract could possibly be through changes in implantation site, and altered hormone levels. The increase in uterine muscle contraction might also be another possible mechanism of abortion.

Antiulcer Activity

In the study [53] aqueous extract of *Drynaria quercifolia* showed protection against gastric lesions in the experimental rats. Aqueous extract of leaves of *D. quercifolia* reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extract for their antiulcerogenic activity.

A study was investigated against pylorus ligation and ethanol induced ulcer models in experimental rats at doses of 250 and 500 mg/kg body weight and the extract of the plant showed significant ($p<0.05$) reduction in gastric volume, free acidity and ulcer index as compared to control. Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity [54].

Antipyretic Activity

The antipyretic effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of *D. quercifolia* was investigated [55]. Intraperitoneal administration of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of *D. quercifolia* at a dose of 80 mg/kg body weight were shown to significantly reduce the elevated body temperature of rabbit, which was compared with standard (aspirin) and solvent used.

Antipyretic activity of *D. quercifolia* rhizome was performed using brewer yeast induced pyrexia test in rats. Fever was induced by injecting 10 ml/kg (s.c) of 20% aqueous suspension of Brewer's yeast in normal saline and rectal temperature was recorded by clinical thermometer before and after 12hrs of yeast administration. *Drynaria quercifolia* at a doses of 100, 250, 500 mg/ kg, showed significant anti-pyretic effect by decreasing the rectal temperature. Among the three concentrations, 500mg of plant extract exhibited remarkable antipyretic activity by decreasing the rectal temperature of rats in 1 hr (38.06oC), 2hr (37.33oC), 3hr (37.09oC) after treatment which was higher than that of standard drug paracetamol (200 mg/kg) (37.240C). This finding demonstrated that *Drynaria quercifolia* have remarkable anti-pyretic activity when compared with positive control and thus have great potential as a source for natural health products [56].

Antidiabetic and Hypolipidemic Activity

The chloroform and ethanolic extract of *D. quercifolia* possesses antidiabetic and hypolipidemic activity in experimental animal models, which support the traditional uses of *Drynaria quercifolia* Linn rhizome. Rhizome in Streptozotocin induced diabetic rats. Glibenclamide (5mg/kg) was used as reference standard for the activity comparison. Ethanolic and chloroform extract of *Drynaria quercifolia* Linn. Rhizome in a dose of 400mg/kg used for the antidiabetic and hypolipidemic study. Fasting blood glucose level and lipid profile parameters were measured, from this result it was concluded that both extract has significant antidiabetic and hypolipidemic property [57].

Anti-arthritic Activity

Drynaria quercifolia was found to have multiple modes of administration for arthritis in ethnomedicine [58]. Anti-arthritic effect of rhizome *D. quercifolia* was studied by assessing the levels of lysosomal enzymes, protein bound carbohydrates, urinary degradative collagen and serum cytokines on control and adjuvant induced arthritis. The paw swelling and body weight were also analyzed. The levels of ROS and lysosomal enzymes in neutrophils of control and adjuvant induced animals were also estimated. The rhizome water extract at doses of 100 and 200 mg/kg reduced the paw thickness and elevated the mean body weight of arthritic rats. The treatment with extract showed a significant reduction in the levels of plasma and liver lysosomal enzymes as well as protein bound carbohydrates and urinary degradative collagen levels. The treatment reduced the levels of ROS and lysosomal enzymes in neutrophils significantly. The significant reduction in the levels of serum pro-inflammatory cytokines (TNF-α and IL-1β) and the increment in the levels of anti-inflammatory cytokine (IL-10) were also observed by the treatment. So the study [59] supports the traditional claim of using *D. quercifolia* to treat rheumatism.

Anti-allergic and Anti-lipidperoxidative

Mast cells are tissue cells which possess granules that contain potent mediators of allergic reactions. The study [60] have reported that degranulation of mast cells cause the release of histamine, acetylcholine, adenosine, neutral peptide, cytokines, chemokines, growth factors and also activates arachidonic acid pathway which enhance the inflammatory process typical of allergic reactions. All these events are involved in allergic conditions like asthma, allergic rhinitis, erythema, pruritis and oedema formation.

In higher animals, lipid peroxidation is known to cause destabilization and disintegration of cell membrane leading to liver injury, arteriosclerosis and kidney damage [61]. Another Reports [62-63] showed that lipid peroxides are pro-inflammatory and can damage the tissues directly. Halliwell [64] has stated that protection against free radical induced lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders which are associated with membrane damage.

According to a study [17], the ethanol extract of rhizomes of *D. quercifolia* (DQ), ethyl acetate extract (EDQ) and hexane extract (HDQ) significantly attenuated degranulation of peritoneal mast cells of Swiss albino mice and showed significant reduction in FeCl2-AA induced lipid peroxidation in rat liver in vitro. The High Performance Liquid Chromatography (HPLC) study showed that naringin was found to be 1.6% in EDQ. Naringenin was found to be 0.53% in DQ and 0.15% in EDQ. The total phenolic content was found to be very high, DQ 244mg/g and EDQ 416mg/g equivalent of gallic acid.

The results suggest potent antiallergic and anti-lipid peroxidative properties of *D. quercifolia* that substantiates its extensive use in ethnomedicine to treat inflammatory disorders.

Anti-inflammatory Activity

Anti-inflammatory activity was evaluated using carrageenan induced rat paw oedema [56]. The rats foot paw become oedemateous after injection of carrageenan. The administration of extract at doses of 100,250,500 mg/kg b.w produced a significant anti-inflammatory activity at 2 ½ hours with paw oedema inhibition of 21%, 33 % and 58% respectively, while the reference drug Dexamethasone inhibited paw oedema of 40 %. Only the extract at the dose of 500 mg showed a maximum inhibition of carrageenan induced rat paw oedema when compared with standard drug.

In-vitro and *in vivo* anti-inflammatory activity were evaluated using albumin denaturation and membrane stabilizing method and carrageenan induced inflammation method [43]. In-vitro cyclooxygenase inhibition was also done to investigate the pathway of anti-inflammatory action. Both methanol (MEDQ) and aqueous (AEDQ) extracts showed significant ($p<0.01$) inhibition of rat paw edema in dose dependent manner and the MEDQ was the most active. The MEDQ exhibited highest inhibition of COX-1 and COX-2, protein denaturation and hemolysis at 100µg/ml. These observations established the traditional claim of usefulness of *D. quercifolia* rhizome against inflammation, which could be due to cyclooxygenase enzyme inhibition and free radical scavenging activities of the extracts.

Anti-nociceptive and Anti-odematous Activity

Drynaria quercifolia produced a significant dose dependent inhibition of granuloma formation. At 500 mg/kg, *D. quercifolia* produced 55.56% inhibition in the exudative phase and 62.83% in the proliferative phase. *D. quercifolia* also significantly attenuated acute and delayed phases of formalin-induced pain and acetic acid- induced writhing episode in mice [24].

The anti-oedematous property of fertile fronds (FF) of *D. quercifolia* may be due to the inhibition of pro-inflammatory mediators, free radical scavenging, or membrane stabilizing effects. The analgesic property of FF may be due to its effect on peripheral nociceptors, spinal mediated central action, or interaction with various receptors including capsaicin receptors. The synergistic action of the phytochemicals present in FF could be the reason for the proposed anti-inflammatory and analgesic effects [65]. According to another study [66], Anti-nociceptive activity was evaluated by acetic acid induced writhing inhibition and radiant heat tail-flick methods. In peripheral method of anti-nociception, the methanolic crude extract (400 mg/kg) and carbon tetrachloride fraction (400 mg/kg) of *D. quercifolia* showed significant anti-nociceptive activity having 40.94% and 45.64% ($P<0.001$) of writhing inhibition respectively compared to standard diclofenac (51.68 % inhibition). The aqueous soluble fraction of the extract (400 mg/kg) also showed promising anti-nociceptive activity having 34.9% of writhing inhibition ($P<0.001$). In the radiant heat tail-flick method of central anti-nociception, the methanolic crude extract (400 mg/kg) and petroleum ether fraction (400 mg/kg) of *D. quercifolia* showed significant analgesic activity having 63.92% ($P<0.001$) and 64.49% ($P<0.01$) elongation of reaction time respectively at 60 minutes after administration of sample compared to the standard morphine (75% elongation). The carbon tetrachloride fraction (400 mg/kg) also demonstrated potent analgesic activity (51.14% elongation). Thus the result of the studies demonstrated anti-nociceptive activities of the rhizomes of *D. quercifolia*.

Anti-Urolithiatic Activity

Urolithiasis is a common urinary tract disorder. Saponins rich constituents of plants may be effective in urolithiasis treatment. From the study of the alcoholic extract of *D. quercifolia,* was effective in *in-vivo* anti-urolithiatic activity on induced calcium oxalate crystals in rats and found noteworthy in treatment of renal calculosis [67].

Cytotoxic Activity

Moderate cytotoxic activity of *Drynaria quercifolia* was also reported by Runa [23] Compared to vincristine sulfate, the LC50 values of crude methanolic extract, chloroform, carbon-tetrachloride, pet-ether and aqueous soluble fractions of *Drynaria quercifolia* leaves were found to be 12.45, 14.95, 13.02, 15.83 and 7.612 µg/ml, respectively. Evaluation of cytotoxic activity of *D. quercifolia* was done using the brine-shrimp lethality bioassay. The carbon tetra-chloride soluble fraction showed the greatest cyto-toxic activity with a LC50 value of 30.31 µg/ml. Petroleum ether, Ethyl acetate and aqueous fractions showed LC50 values of 2,380 µg/ml, 569.39 µg/ml and 41,041.30 µg/ml respectively compared to that of 0.544 µg/ml of vincristine sulfate [42].

The LC50 values for pet-ether, chloroform and ethyl acetate extracts of the rhizomes of D. quercifolia and ampicillin tri-hydrate were found to be 22.0, 16.5, 16.5 and 11.7 µg/ml, respectively.
The extracts were less toxic with higher LC50 values than ampicillin trihydrate. Amongst the extracts, chloroform and ethyl acetate extracts were more cytotoxic with lower LC50 value than pet ether extract. Preliminary phytochemical screening on these extracts revealed the presence of sterol and alkaloid type compound present in the pet ether and chloroform extracts and sterol, alkaloid and polyphenolic type compounds present in the ethyl acetate extracts. According to these results, there is a good probability that metabolites of these plants may have anticancer, antiviral, insecticidal or pesticidal activities [2].

Hepatoprotective Activity
Hydroalcoholic extract of *Drynaria quercifolia* fronds (Dq), its fractions and isolated compound (Dq-4) from ethyl acetate (EA) fraction has been evaluated for hepatoprotective effect [68]. The toxicant CCl4 (1ml/kg) was administered on 4th and 5th day to induce hepatotoxicity in Wistar rats (in-vivo) and the in-vitro hepato-protection was evaluated against CCl4 (1%) induced toxicity in HepG2 cell lines. The pre-treatment of rats with Dq extract, EA fraction and Dq-4 for 7 days produced a significant dose dependent hepatoprotective action by decreased levels of hepatic enzymes, total bilirubin and TBARS and increased levels of total proteins, albumin, and reduced glutathione. The histological examination provided the supportive evidences. Additionally, Dq extract, EA fraction and Dq-4 significantly decreased the CCl4-induced in-vitro toxicity in HepG2 cellines evident by MTT reduction assay and trypan blue method.

Mosquito Repellent Activity
The Rhizome of *Drynaria quercifolia* extracts exhibit high repellency (as high as 90 to 100%) to adult female mosquito species *C. quinquefaciatus* and *Aedis aegypti* with increase in concentration of 160mg, 170mg and 180mg of AQ, DCM, MET. PET 500 ppm extracts concentration of rhizome showed significant decrease in the larva population of same spp. as compared to other three extracts namely DCM, MET and PET. PET extract is very effective and it showed the 'Knock down' effect within 20 min at 160 mg [69].

Pesticidal and Pest Repellency Activity
Pests/insects often cause extensive damage to stored grain products, which is a serious problem throughout the world. Jacobson [70] and Ketkar [71] have reviewed the effectiveness of plant derivatives for use against grain pests. But only a small number of pest control products directly obtained from plants [72-73].
Among petroleum ether, ethyl acetate, chloroform and methanol soluble fractions of ethanol extract of rhizome of *D. quercifolia*, only the chloroform soluble fraction showed significant pesticidal activity against the pest. Furthermore, the fraction also showed significant pest repellency activity against the pest. Isolated compound 3,4-dihydroxybenzoic acid was inactive against the pest. Overall, it can be stated that good pesticidal and pest repellency activities of the rhizome of *D. quercifolia* suggesting its suitability as botanical pesticide in controlling *T. castaneum* of stored food commodities [74].

Thrombolytic Activity
The thrombolytic activity of methanolic extracts of *Drynaria quercifolia* were fractionated by the modified Kupchan partitioning method to render pet-ether soluble fraction (PESF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF) and aqueous soluble fraction (AQSF). To observe their thrombolytic potential, a prompt and swift method was involved where streptokinase and water were used as positive and negative control, respectively. AQSF and PESF of *D. quercifolia* exhibited highest thrombolytic activity by clot lysis of 34.38%, 34.27% respectively and showed significant percentage (%) of clot lysis compared to standard streptokinase (41.05%) while the negative control water revealed 3.31 % lysis of clot [75].

Neuropharmacological Activity
Drugs acting in the central nervous system are still the most widely used group of pharmacological agents [76]. The neuropharmacological effect of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of Drynaria quercifolia were studied in mice by intraperitoneal administration. The tests used were determination of effect on duration of diazepam-induced sleep, determination of effect on nikethamide-Induced toxicity, light dark test and force swimming test. The duration of diazepam-induced sleep was extended by administration of these fractions. Nikethamide at high dose cause death of mice and time to cause death of mice was delayed by administration of these fractions. In light dark test and force swimming test, these fractions were given diazepam type effect. These results suggest that both these fractions of *D.*

quercifolia rhizome have dose dependent depressant effect [11].

Wound Healing Activity

Wound healing is a process of universal recurring phenomenon in animal systems, comprises of different orderly phases [77-79] that restores cellular structures and tissue layers of the injured tissue intact. Wounds (both incision and excision type) were created on Swiss albino rats of Wister strain [80]. Wound micro flora was screened from the excision model on respective days. Pure culture isolations of the organisms were tested for their identification and the same organism was taken as the test organism in the respective antimicrobial study against methanol extract used in the ointment base. Wound micro flora isolation and wound healing efficacy was also studied in the diabetes induced rats at parallel. Methanol and Chloroform extracts of *D. quercifolia* were found to have significant healing potential evident from reduction in wound size, epithelization time and the reason were also supported by phytochemical and microbial studies. In addition, triterpinoids reported to possess ability to increase the collagen content which is one of the factors promoting wound healing [81]

Acute Toxicity Study

The solvent extracts of *D. quercifolia* did not show mortality at the dose of 2000 mg/kg body weight [80]. The tested extracts were administered orally and animals were observed closely for first 2 hours for any toxic activity like motor activity, salivation, coma and death. Common side effects such as, mild diarrhea, loss of weight and depression in treated group of animals were not recorded within 7 days of observation. A histopathological studies of isolated 3, 4-dihydroxybenzoic acid from *D. quercifolia* has shown no significant effect on liver, Kidney, heart and lung of mice. The results of the sub-acute toxicity studies indicate its safety for clinical trials [27].

Pharmacological Activities of Drynaria

Rhizomes extracts of some *Drynaria quercifolia* species are used extensively in South Asia and Maritime Southeast Asia and various ethnopharmacological studies have been conducted into the properties of Drynaria [82-84]. Several studies have shown that basket ferns (*D. roosii* in particular) are effective in preventing resorption of bone cells and osteoporosis, increases bone density, and have therapeutic effects on bone healing [85-88]. Drynaria has been shown in multiple studies to stimulate [89-92] and osteoblasts to produce more healthy bone tissue [89-103]. Anti-osteoporotic effects of extracts of Drynaria have been shown in female mammals following the loss of ovaries [89, 104]. An animal study done by one group of researchers revealed that the extract of Drynaria suppressed bone resorption and promoted bone [105].

In 2011, scientists from School of Medicine in Hangzhou observed a significant decrease in the number of bone dissolving cells (Osteoclasts) in the Drynaria treated group as compared to the control animals. Their study has shown positive effects on improving alveolar bone remodeling and the study concluded that Drynaria could be a alternative medicine for periodontal therapy based on its ability to reduce alveolar bone resorption while supporting the bone-building effects of osteoblasts [106]. Other recent study [107] found that Drynaria effectively increased the number of osteoblasts and reduced the number of osteoclasts, providing an overall osteogenic effect in female SPF Wister rats.

The action of Drynaria extracts on the periodontal ligament are very important directions of research for natural dental health because periodontal ligament serves as a source for regenerating bone tissue, reversing of these ligaments during gum loss is central to halting and preparing periodontal disease [94, 108, 109, 110]. Naringin, one of the active ingredients in Drynaria, stimulates protein synthesis and metabolism of human periodontal ligament cells [94] and naringin, also can promote proliferation of human periodontal ligament cells by enhancing alkaline phosphatase (ALP) activity which is an important osteoblast function in bone building [108,111].

Total flavonoid content of Drynaria rhizomes has the ability to promote osteogenic differentiation of bone marrow cells into bone-building osteoblast cells at different concentrations of exposure to glucose. The study done by Chinese researchers [112] revealed that Drynaria counters this damage at the highest concentrations of glucose and concludes that the flavonoids may help as a therapy for diabetic osteoporosis.

Conclusions

According to the above discussions about assorted bio-evaluations of *Drynaria quercifolia*, it can be said that *D. quercifolia* has a wide range of bioactive constituents those could be useful for curing of many diseases. Especially the Genus, *Drynaria* has widely been shown to improve bone rebuilding in several

examples of excessive bone loss. Early scientific research on *Drynaria quercifolia* already demonstrated its *in-vivo* and *in-vitro* ability to evaluate significant clinical activities to combat diseases.Further scientific studies should be done to explore new promising drugs and this review is an effort to get a comprehensive view and to compile all major findings of the pharmacological activities including phytochemical constituents of *D. quercifolia* (L.) J. Smith.

Competing Interest

The author (s) declare they have no financial and non-financial competing interest.

Authors' Contributions

All the authors has been contributed equally while preparing the review manuscript. All the authors read and edited the manuscript for the final submission.

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Table 1. Classification (Taxonomy) of the D*rynaria quercifolia*

Rank	Scientific name and Common name
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Petridophyta
Class	Filicopsida
Order	Polypodiales
Family	Polypodiaceae
Genus	*Drynaria* (Bory) J. Sm.
Species	*Drynaria quercifolia* (L.) J. Sm.

Table 2. Results of phytochemical screening of different extracts of *D. quercifolia* leaves [23]

Group of phytoconstituents	MEF	CTSF	CSF	PTSF	AQSF
Alkaloids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Glycosides	-	+	-	-	-
Saponins	+	-	-	+	+
Phytosterols	-	+	+	+	-
Phenols	+	+	+	+	+
Tannins	-	+	+	+	+
Flavonoids	+	-	+	+	+
Proteins and amino acids	-	+	+	-	-
Fats & fixed oils	-	-	-	-	-
Gum and mucilages	-	-	-	-	-
Triterpenes	-	+	+	+	+

Here, MEF= Methanolic extract fraction, CTSF= Carbon tetrachloride soluble fraction, CSF=Chloroform soluble fraction, PTSF= Petroleum ether soluble fraction, AQSF= Aqueous soluble fraction

Table 3. Quantitative chemical analysis of different solvent extract of *Drynaria quercifolia* [25]

Tests	Extracts			
	Petroleum ether	Chloroform	Methanol	Water
ALKALOIDS				
Mayer's test	_	+	+	+
Wagner's test	_	+	+	+
Dragendorff's test	_	+	+	+
Hager's test	_	+	+	+
GLYCOSIDES				
Mod. Borntrager's test	_	_	_	_
Legals test	_	_	_	_
Libermann-Buchard's test	+	+	+	_
aljet test	_	_	_	_
SAPONINS				
Forth test	_	_	-	-
PHYTOSTEROLS				
Libermann-Buchard's test	+	+	+	_
PHENOLICS AND TANNINS				
Ferric chloride test	_	_	+	+
Gelatin test	_	_	_	_
Lead acetate test	_	_	+	+
Alkaline reagent test	_	_	_	_
Shinoda test	_	_	_	_
Vanillin Hcl test	_	_	_	_
PROTEINS AND AMINO ACIDS				
Million's test	+	_	+	+
Biuret test	_	_	_	_
Ninhydrin test	+	_	+	+
FIXED OILS AND FATS				
Stain test	+	_	_	_
Soap test	+	_	_	_
CARBOHYDRATES				
Molisch's test	_	_	+	+
Benedict's test	_	_	+	+
Barfoed's test	_	_	+	+
GUMS AND MUCILAGE				
Alcohol ppt test	_	_	_	+
Ruthenium test	_	_	_	+