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Therapeutic and pharmacological aspects of photodynamic product chlorophyllin

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ABSTRACT: Medicinal plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. They can provide biologically active molecules and lead structures for development of modified derivatives with enhanced activity or reduced activity. The isolation and identification of active principles and elucidation of the mechanism of action of a drug is of paramount importance. One such compound is chlorophyllin, a water soluble analogue of the ubiquitous green pigment chlorophyll. It acts as an effective inhibitor of aflatoxin hepatocarcinogenesis in animal models by blocking carcinogen bioavailability. Further anti-cancer effects of chlorophyllin including antioxidant activity, inhibition of enzymatic activity that converts inert procarcinogens into active carcinogens, stimulation of enzymatic activity that promotes the elimination of toxic substances from the body and antitumor activity have likewise been evidenced by controlled studies. Phytotherapy of snails by photodynamic chlorophyllin is a new approach to control the epidemic fasciolosis. Photosensitive chlorophyllin is degraded very fast without the formation of toxic byproducts, therefore, it is environmentally sound and economically safe also.

Keywords: Spinach; Chlorophyllin; Therapeutic effect; Photodynamic product; Chlorophyll; Medicinal plant.

1. INTRODUCTION

Medicinal plants are the ‘backbone’ of traditional medicine which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis [1]. Plants are sources of life saving drugs and have been used for medical treatment in human history [2]. Evidences exists that traditional systems of medicine continue to be widely practiced on many accounts population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [3]. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes, including treatment of infectious diseases [4].

Reports on traditional medicinal uses of chlorophyll in alternative forms of medicine are known since ages. Now-a-days chlorophyll has been used in the field of medicine as remedy and diagnostics. Chlorophyllin possess therapeutic importance due to its antimutagenic and anticarcinogenic [5], antioxidative [6] and antihyperglycemic effects [7] in different experimental systems. This article enumerates therapeutic claims of chlorophyll as drugs based on investigative findings of modern science. A brief overview of research and developments of medicinal uses of chlorophyll will be presented in this review along with challenges of potential applications of chlorophyll and its derivatives as pharmacological agents.

2. SPINACH (*Spinacia oleracea*)

Taxonomical classification:
Kingdom: Plantae
Order: Caryophyllales
Family: Amaranthaceae
Genus: *Spinacia*
Species: *oleracea*

3. BOTANICAL DESCRIPTION

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae native to central and western Asia. It is an annual plant which grows up to 30 cm tall. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, ovate to triangular and very variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. Chlorophyll is present in green leafy vegetables and reaching levels as high as 5.7% in Spinach [8].

4. CHLOROPHYLL

Chlorophyll (Fig. 1) is an important pigment in the process of photosynthesis and found in all photosynthetic organisms including plants, blue-green algae and eukaryotic algae [9]. It is a tetrapyrrole compound containing a central Mg^{2+} ion and an isoprenoid phytyl side chain [10]. There are 6 different chlorophylls that have been identified namely a, b, c, d, e and f [11].

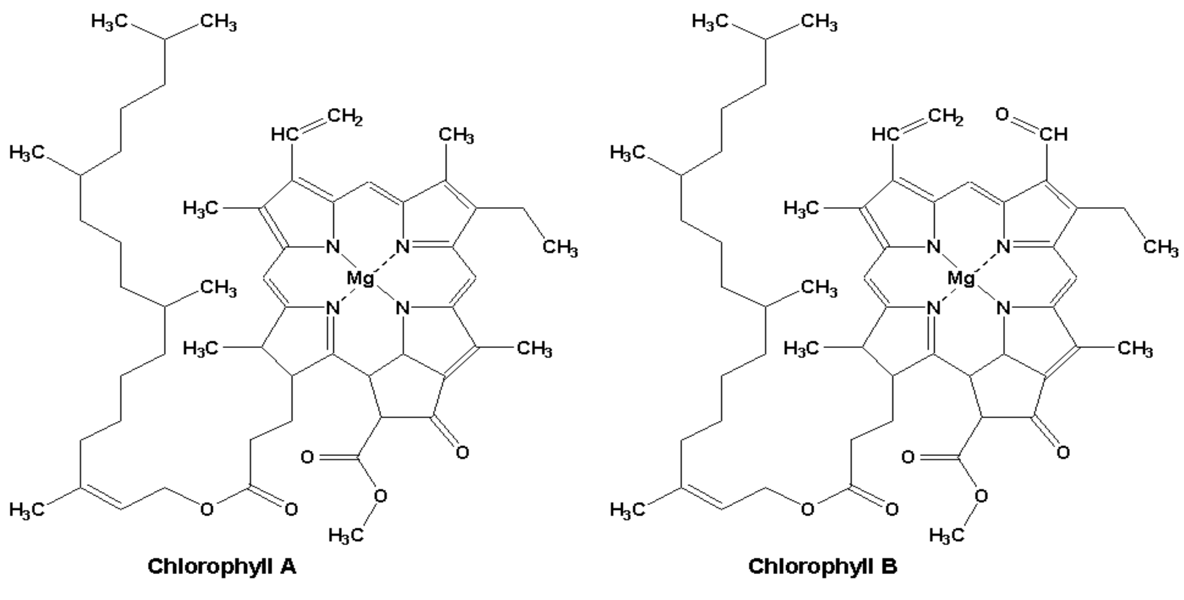

Figure 1. Structures of chlorophyll A and chlorophyll B.

Chlorophyll a and chlorophyll b are the two major types of chlorophyll and differ only in the composition of one of their structural side chains. Chlorophyll a contains $–CH_3$ group and chlorophyll b contains –CHO group (in a position C-7) [12]. The small difference in one of the side chains allows each type of chlorophyll to absorb light at slightly different wavelengths. Greenish-yellow chlorophyll a is the most prevalent type of chlorophyll. It is principal photosynthetic pigment and found in plants, algae and other aquatic organisms. The ratio of chlorophyll a to chlorophyll b in the chloroplast is 3:1 [13]. The empirical formula of chlorophyll a is $C_{55}H_{77}O_5N_4Mg$ and chlorophyll b is $C_{55}H_{70}O_6N_4Mg$. Chlorophyll b is an accessory photosynthetic pigment with olive-green color. It is mainly found in land plants, aquatic plants and green algae [11]. Chlorophyll c is found in diatoms, dinoflagellates and brown algae. Chlorophyll d is a minor pigment and present in red algae. Chlorophyll e is a very rare type of chlorophyll that is found in some golden algae. Chlorophyll f was recently discovered in some cyanobacteria near Australia. All kinds of chlorophyll are fat-soluble [14].

5. CHLOROPHYLL DERIVATIVES

Chlorophyll, a porphyrin derivative, is a major photosynthetic pigment. Chlorophyll is often accompanied by the presence of its various derivatives. It can be degraded to form a number of compounds including (i) pheophytin, a chlorophyll derivative lacking Mg^{2+}, (ii) chlorophyllin, a chlorophyll derivative lacking phytyl group and is formed by chlorophyllase-mediated hydrolysis and (iii) pheophorbide, a chlorophyll derivative lacking both Mg^{2+} and phytyl groups [15].

Chlorophyllin is a semi-synthetic mixture of sodium copper salts derived from chlorophyll [16]. During the synthesis of chlorophyllin, the magnesium atom at the centre of the ring is replaced with copper and the phytol esters are replaced with sodium, making it soluble in water [16]. As a result of these changes, chlorophyllin is more stable than chlorophyll [17]. Its most common form is a sodium/copper derivative used as a food additive and in alternative medicine [18]. As a food coloring agent, copper complex chlorophyllin is known as natural green 3 and has the E number E141. Trisodium copper chlorine e_6 and disodium copper e_4 are two compounds commonly found in commercial chlorophyllin mixtures.

6. PHOTODYNAMIC PRODUCT CHLOROPHYLLIN

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main source of drugs. Now a days use of plants products are acceptable due to their wide range of ideal properties, such as high target toxicity, low mammalian toxicity, low cast, solubility in water and biodegradability. Chlorophyllin is a semi-synthetic derivative of the natural green pigment chlorophyll [16]. Unlike natural chlorophyll, chlorophyllin is water-soluble [19, 20, 21]. It can simply extracted from different plant resources (e.g. spinach, grass, dandelion, green cabbage, water hyacinth, algae etc.) [19, 70, 72]. It displays some technological advantages over chlorophyll, such as greater hydrophilicity and tinctorial power and higher stability towards acid and light. Natural chlorophyll and its derivates can easily be extracted, processed and offers an inexpensive option for controlling vectors of parasites, which would be advantageous especially for developing regions of the world [22].

7. PREPARATION OF EXTRACTED CHLOROPHYLLIN

Preparation of chlorophyllin was done according to the method of Wohllebe et al. [23] as modified by Singh and Singh [20]. Chlorophyll was isolated from spinach (*Spinacia oleracea*) using 100% ethanol (for about 2h at 55ºC). Then, $CaCO_3$ (about 1 mg/g plant material) was added as a buffer, it prevent the transformation of chlorophyll into pheophytin. Before adding petroleum benzene the extract was irradiated with solar radiation for 1-2h. The extract was subsequently filtered using Whatman qualitative filter papers (Whatman International Ltd, UK) and 50 ml petroleum benzene was added. After addition of benzene the mixture was well shaked as a result the chlorophyll moved into the lipophilic benzene phase. The two phases were separated in separatory funnel and about 1.0 ml methanolic KOH was added to 50 ml of the benzene phase. Upon agitation the chlorophyll came into contact with the methanolic KOH and was transformed into water-soluble chlorophyllin. (This process occurs due to the breakage of the ester bond between the chlorophyllin and the phytol tail by saponification). After separation of the methanolic KOH phase and the benzene phase most of the chlorophyllin was found in KOH phase. The extract was stored in a dark flask at room temperature. However, only fresh chemicals were used in the course of these experiments.

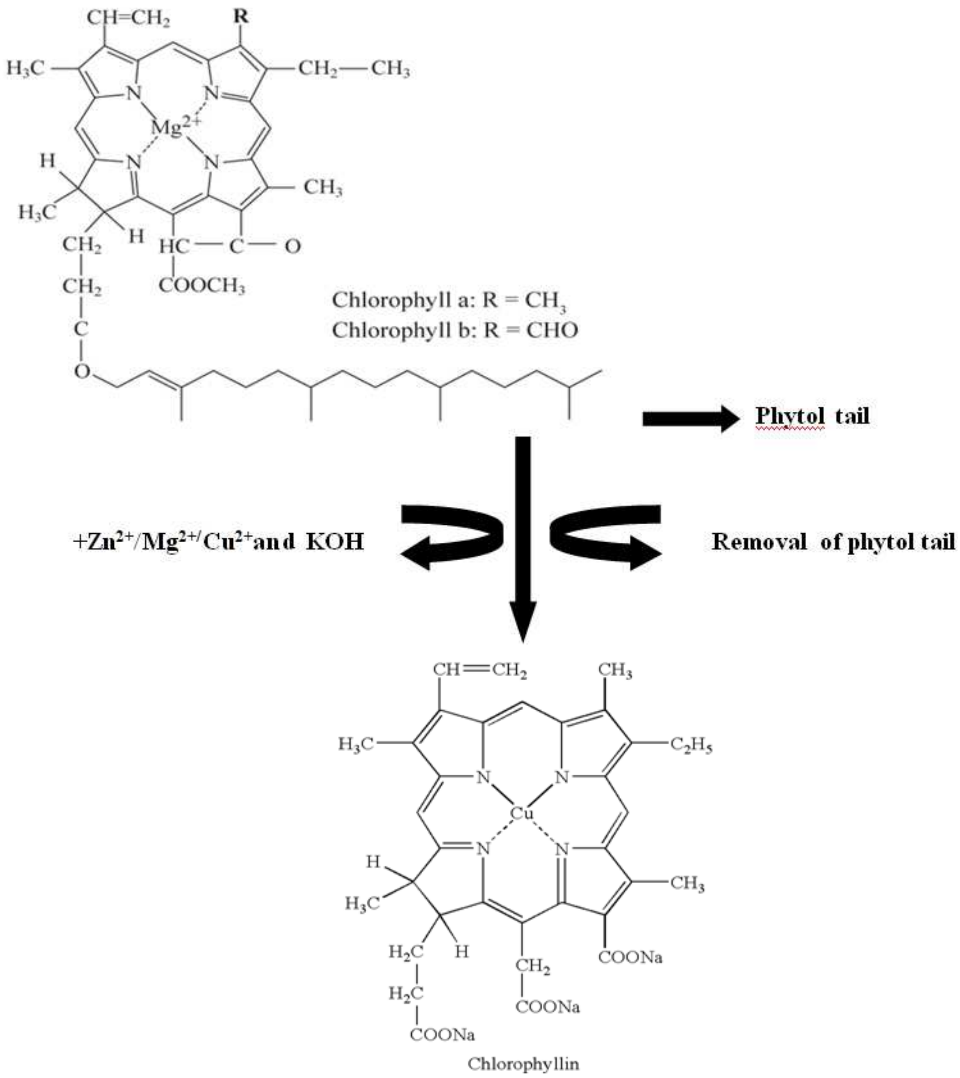

Figure 2. Transformation of chlorophyll in chlorophyllin from spinach (*Spinacia oleracea*).

8. MECHANISM OF PHOTODYNAMIC THERAPY (PDT)

The term 'Photodynamic' was coined by Von Tappeiner in 1904 to describe oxygen-dependent chemical reactions induced by photosensitization. In general, photosensitization-based therapy (PDT) is a treatment modality involving the administration of a photosensitizing compound, which selectively accumulates in the target cells, followed by local irradiation of the lesion with visible light. The combination of two absolutely nontoxic elements, i.e. drug and light, in the presence of oxygen results in the selective destruction of tissue. The expanding use of PDT is based on the pioneering work of [24], who presented extensive data on the successful application of this novel technique for the treatment of cancer in 1978. Intensive clinical research culminated in the approval of PDT for the management of selected malignancies in Canada, Japan, France, the Netherlands, Germany and the United States [25]. Now, the question arises inevitably: how does photodynamic therapy work?

It is a result of the combined effect of three non-toxic agents: photosensitizer, light and oxygen [26].

8.1. Photosensitizers

A large number of photosensitizing drugs have been used *in vitro* and *in vivo* treatment. The physico-chemical properties of the photosensitizer are important for the efficacy of photosensitization. Chemical purity, capability to localize specifically in neoplastic tissue, short time interval between the administration of the drug and its maximal accumulation in hyperproliferating tissue, rapid clearance from normal tissues, activation at wavelength with optimal tissue penetration, high quantum yields for the generation of singlet oxygen and lack of dark toxicity are desirable features of an ideal photosensitizer. Hematoporphyrin derivative (HPD) was the first systematically studied photosensitizer for clinical PDT. Chlorophyll derivatives used as a photosensitizer in experimental and clinical photodynamic therapy applications.

8.2. Light sources

Photosensitization has been performed with the help of different types of light sources. Metal halogen lamp, which emits 600-800 nm radiation at high power density, short-arc xenon lamp, tunable over a bandwidth between 400-1200 nm. Lasers also provide the exact selection of wavelengths and the precise application of light such as the gold vapor laser (GVL) and the copper vapor laser- pumped dye laser (GVDL), produce brief light pulses of millisecond to nanosecond duration. But they are expensive, relatively immobile and require frequent repair. To remove these disadvantages the development of semiconductor diode lasers is a novel approach. Portable diode lasers, such as the gallium-aluminium-arsenide laser, produce light in the range from 770 to 850 nm, which corresponds to the absorption peaks of many new photosensitizers.

8.3. Oxygen

The efficacy of photosensitization is directly related to the yield of $^1O_2$ depends on the concentration of oxygen in the tissue [27]. Hypoxic cells are very resistant to photosensitization and the photodynamic reaction mechanism itself may consume oxygen at a rate sufficient to inhibit further photosensitization effects. It has been suggested that hyperbaric oxygen might enhance the photosensitization effect.

Chlorophyll derivates such as chlorophyllin is a photodynamically active substance [19]. The conversion of hydrophobic chlorophyll into water soluble chlorophyllin is technically not demanding [22]. Due to water solubility, chlorophyllin can be applied in aquatic environments. In general, photodynamic substances (^3P) are not toxic in darkness but are activated by light [28], and transformed to a reactive triplet state T1. Upon reaction with oxygen ($^3O_2$) reactive singlet oxygen is produced ($^1O_2$), which has highly cytotoxic effects [29].

$$^3P + {}^3O_2 = P + {}^1O_2$$

Photosensitizers are the molecules which are excited by light [30]. The excited state can react with other molecules changing the chemical properties of the reaction partner. Photodynamic reaction with oxygen leads to the formation of the highly reactive singlet oxygen, which can react with various biomolecules [31]. In addition, photosensitizers such as chlorophyll derivates are capable to oxidize and reduce other molecules. In the excited state chlorophyll is a strong reductant, which can transfer electrons onto other molecules, but in the subsequent oxidized state chlorophyll is a strong oxidant, which may oxidize other biomolecules. As a result, reactive oxygen species (ROS), such as superoxide or hydrogen peroxide are formed posing strong oxidative stress to the cells. Excessive oxidative stress result in damage to cell membranes, proteins, DNA and other cell structures [21, 32].

9. PRESERVATION AND STABILITY OF CHLOROPHYLLIN

Chlorophyll derived from natural sources undergoes rapid degradation during storage, but their stability can be increased by de-esterification or by the substitution of the central metal atom with Cu or Zn [33]. However, the choice of Cu may not be safe from an ecosystem point of view. In addition to stability, photodynamic activity after long-term storage is another challenge. However, this problem can be resolved by lyophilization (freeze drying) of chlorophyll derivatives immediately after isolation [34]. For example, chlorophyllin lyophilized after isolation was tested for its stability and photodynamic efficiency for 30 days and the lyophilized chlorophyllin was stable and photodynamically active against larvae even after 30 days [34]. Interestingly, lyophilized chlorophyllin was even more effective than the freshly isolated non-lyophilized chlorophyllin or chlorophyllin preserved in methnol solution [34]. Thus, lyophilization ensures long-term stability of chlorophyllin and increases its photodynamic activity.

10. ADVANTAGE OF PHOTOSENSITIZER CHLOROPHYLLIN

Chlorophylls, found in green plants, are natural, fat-soluble. The chlorophyll derivates chlorophyllin is a semi-synthetic mixture of water-soluble sodium copper salts [16]. There are several advantages of chlorophyll derivatives such as:

10.1. Antioxidant effects

Chlorophyllin can neutralize several physically relevant oxidants in vitro [35] and limited data from animal studies suggest that chlorophyllin supplementation may decrease oxidative damage induced by chemical carcinogens and radiation [36]. The antioxidant effect of chlorophyllin in splenic lymphocytes in mice has been observed [37]. Recently, it have been demonstrated that chlorophylls and pheophytins act as antioxidants to prevent oxidative DNA damage and lipid peroxidation both by chelating reactive ions and by scavenging free radicals [38]. The anti-oxidant activity and antimicrobial activity of chlorophyllin from *Mimosa pudica* was evaluated early [39]. Recently, it has been observed that chlorophyllin possesses antioxidative activity and has the potential to ameliorate diabetes associated oxidative stress in mice [40].

10.2. Modification of the metabolism and detoxification of carcinogens

Because chlorophyll does not dissolve in water, food sources of chlorophyll do not bind to mutagenic substances to a significant extent. Chlorophyllin, being water-soluble, can significantly bind to environmental mutagens such as the polycyclic aromatic hydrocarbons benzo[a]pyrene [41], and dibenzo{a,i}pyrene [8]. Chlorophyllin binds to mutagens twenty times better than resveratrol and thousands of times better than xanthines [42]. *In vitro* studies indicate that chlorophyllin may decrease the activity of cytochrome P450 enzymes [43]. Phase II biotransformation enzymes promote the elimination of potentially harmful toxins and carcinogens from the body. Limited data from animal studies indicate that chlorophyllin may increase the activity of the phase II enzyme, quinone reductase [44]. A new study demonstrated that chlorophylls mediate changes of the redox status of pancreatic cancer cells which might partially be responsible for their anticancer effects and also contribute to reduce the occurrence of cancer among consumers of green vegetables [45].

10.3. Therapeutic effects

A recent study showed that human colon cancer cells undergo cell cycle arrest after treatment with chlorophyllin [46]. The mechanism involved inhibition of ribonucleotide reductase activity. Ribonucleotide reductase plays a pivotal role in DNA synthesis and repair, and is a target of currently used cancer therapeutic agents, such as hydroxyurea [46]. This provides a potential new avenue for chlorophyllin in the clinical setting, sensitizing cancer cells to DNA damaging agents. Chlorophyll-a is a novel photosensitizer and recently its clinical efficacy and safety was used for acne treatment and it was suggested that chlorophyll-a photodynamic therapy for the treatment of acne vulgaris can be effective and safe with minimal side effects [47]. The effect of sodium copper chlorophyllin complex was also examined [48]. It was reported that chlorophyllin have the potential to repair the photoaged skin by stimulating the biomarkers in human extracellular matrix. Chlorophyllin-M is a new photosensitive compound which is derived from chlorophyll.

Earlier experiments on rabbit prove that chlorophyllin-M may become a new cost-effective agent in the retinal therapeutic arsenal [49].

10.4. Wound healing

Chlorophyllin has been used orally as an internal deodorant and tropically in the treatment of slow-healing wounds for more than 50 years without any serious side effects. During the late 1940s and 1950s, a series of largely uncontrolled studies in patients with slow-healing wounds, such as vascular ulcers and pressure (decubitus) ulcers, reported that the application of topical chlorophyllin promoted healing more effectively than other commonly used treatments [50]. In the late 1950s, chlorophyllin was added to papain and urea-containing ointments used for the chemical debridement of wounds in order to reduce local inflammation, promote healing, and control odor [51]. Recently, a spray formulation of the papain/urea/chlorophyllin therapy has become available [52].

10.5. Internal deodorant

Several case reports have been published indicating that oral chlorophyllin (100-300 mg/day) decreased subjective assessments of urinary and fecal odor in incontinent patients [51]. Trimethylaminuria is a hereditary disorder characterized by the excretion of trimethylamine, a compound with a “fishy” or foul odor. A recent study in a small number of Japanese patients with trimethylaminuria found that oral chlorophyllin (60 mg three times daily) for three weeks significantly decreased urinary trimethylamine concentrations [53]. Oral preparations of sodium copper chlorophyllin (also called chlorophyllin copper complex) are available in supplements and as an over-the-counter drug (Derifil) used to reduce odor from colostomies or ileostomies or to reduce fecal odor due to incontinence. Sodium copper chlorophyllin may also be used as a color additive in foods, drugs, and cosmetics [28].

10.6. Complex formation with other molecules

Chlorophyll and chlorophyllin are able to form tight molecular complexes with certain chemicals known or suspected to cause cancer, including some heterocyclic amines found in cooked meat [54], aflatoxin-B_1 [55] and polycyclic aromatic hydrocarbons found in tobacco smoke [56]. Supplementation with chlorophyllin before meals substantially decreased a urinary biomarker of aflatoxin-induced DNA damage in a Chinese population at high risk of liver cancer due to unavoidable, dietary aflatoxin exposure from moldy grains and legumes [57]. Scientists are hopeful that chlorophyllin supplementation will be helpful in decreasing the risk of liver cancer in high-risk populations with unavoidable, dietary aflatoxin exposure [58]. However, it is not yet known whether chlorophyllin or natural chlorophylls will be useful in the prevention of cancers in people who are not exposed to significant levels of dietary aflatoxin.

10.7. Photodynamic chlorophyllin acts as a pesticide

Water soluble chlorophyllin exerts pronounced photodynamic activity. Chlorophyllin is a latent remedy against mosquito larvae and aquatic stages in the life cycle of parasites as well as against ectoparasites in fish. Abdel-Kader [59] from the National Institute of Laser Enhanced Science (NILES) had the idea to treat pest organisms photodynamically by means of hematoporphyrin. In different experiments it could be shown that *Culex* larvae were killed in the presence of hematoporphyrin and sunlight. The effectiveness of hematoporphyrin against *Culex* and eggs of the snail *Lymnaea natalensis,* which is a vector of *Fasciola hepatica* was observed [60]. A concentration of about 0.07 µmol/ml in the water was reported to be sufficient to induce photodynamic mortality of the larvae. As hematoporphyrin is too expensive for utilization on a large scale, chlorophyll was considered to be a very economical alternative. Chlorophyll derivative like chlorophyllin has been reported as effective natural photosensitizers against larvae of several insects, flies, moaquitoes and fishes etc. [19, 22, 23]. Different photosensitizers like furocoumarins, thiophenes, phenothiazines, porphyrins, tetraethynylsilanes, xanthenes and rose Bengal have been found effective in killing larvae of insects including mosquitoes [61, 62]. Photosesitizer were also successfully tested against mosquito larvae or as general pesticides (reviewed by Amor and Jori [61]). The effect of different photosensitizer on *Aedes* and *Culex* larvae was observed [62]. Photodynamic properties of chlorophyllin in *Dipter larvae* was intensively investigated [19]. After accumulation of chlorophyllin in intestine, light treatment resulted in high mortality. The LD_{50} dose of externally applied chlorophyllin (addition to the water body) was about 6.88mg/l in *Culex* larvae and about 24 mg/l in *Chaoborus* larvae [19]. Likewise, chlorophyllin concentrations have been measured to kill economically important fish parasites. For the first time the efficiency of the photodynamic substance chlorophyllin to kill different life stages of the protozoan parasite *Ichthyopthirius mulftifiliis* as well as isolated trophonts at low concentrations was observed [63]. Chlorophyllin have been tested to eliminate mosquito larvae as vectors for human illnesses such as malaria, dengue, yellow fewer and others [19, 23, 34].

It was reported that water soluble chlorophyllin (resulting from chlorophyll after removal of the phytol) and pheophorbide (produced from chlorophyllin by acidification), when used at low concentrations and added to the water body, were able to kill mosquito larvae and other small animals within a few hours under exposure of solar radiation [19]. The LD_{50} dose of externally applied chlorophyllin (addition to the water body) was about 6.88 mg/l in *Culex* larvae and about 24 mg/l in *Chaoborus* larvae [19]. In the course of a Malaria Vector Control Program (MVCP) the Innovative Research and Development Corporation (InRaD, Egypt) has performed successful field tests in Nigeria using chlorophyllin. It was reported that exclusively mosquito larvae were killed in a treated pond while all the other water organisms were not affected. The photodynamic toxicity of chlorophyll derivatives against larvae under laboratory conditions is supported by a few field trials. For example, in Kasangati and Namanve cities of Uganda, chlorophyll derivatives were applied on 250,000 m^2 of infected swamps and sand pits. A 0.1-100 μM concentration of chlorophyll derivatives killed 85-100% of *Anopheles gambiae* larvae [64].

In 2009, Erzinger and Hader developed and patented at INPI (National Institute of Intellectual Property), a new Bioinsecticide Nontoxic Biodegradable from a new semi-synthetic derivative of chlorophyll and in conjunction with a formulation system they were able to get a product with high stability front light and maintained the same lethal power of chlorophyll and chlorophyllin for mosquito larvae [65]. New perspectives are developed for the control of mosquito larvae using chlorophyll derivates as photosensitizers [66]. Most experiments were performed with larvae of *Culex* spp. and *Chaoborus crystallinus*. The latter is not a biting insect, but due to its transparency an ideal model organism in order to monitor the *in vivo* uptake of chlorophyllin by light and fluorescence microscopy. It was observed that uptake of chlorophyllin by larva had the drastic effect such as apoptosis and necrosis [23]. New studies have been performed in the field of photosensitizer chlorophyllin to control parasites in aquatic ecosystems [67]. Treatment of ichthyophthiriasis with photodynamic chlorophyllin has been also studied [22].

A number of research works proves that chlorophyllin acts as a potent molluscicide. The toxic effect of chlorophyllin against *L. acuminata* in the presence of red light and sunlight was observed [68]. It has been reported that the combination of monochromatic visible light with chlorophyllin shown effective larvicidal activity against *F. gigantica* [20]. The treatment of photodynamically active chlorophyllin in solar light or in different wavelengths of visible light has significant toxicity effects on vector snail *L. acuminata* [69]. It was already shown earlier that phytotherapy of chlorophyllin formulations against *Fasciola gigantica* infected *L. acuminata* under sunlight exposure was highly toxic against redia and cercaria larvae [70]. Chlorophyllin shows strong anti-reproductive activity against snail *L. acuminata* [21]. It was observed that chlorophyllin bait and red light reduce reproduction capacity in snails [71]. Early, the photodynamic activity of chlorophyllin has been observed against snail *Indoplanorbis exustus* in visible spectral band [72] and snail *Lymnaea acuminata* [73]. HPLC study avowed that molluscicidal activity of chlorophyllin is due to their active components i.e. chlorophyllin a and b [72]. The effects of chlorophyllin on certain biochemical parameter in

L. acuminata were also observed [74]. Recently, a new study has shown the effect of chlorophyllin on snail *Biomphalaria alexandrina* and *Schistosoma mansoni* larvae [75].

11. CONCLUSION

Medicinal plants have been used in virtually all cultures as a source of medicine long before prehistoric period. Assurance of safety, quality and efficacy of medicinal plants and herbal products has now become a key issue in developing countries. Photodynamic chlorophyllin exemplify an immense therapeutic effect and have a great potential in the field of pharmaceuticals. Chlorophyll is readily available almost everywhere, its isolation and further processing are a relatively easy task. The potential of chlorophyll and its derivatives to control parasites and pest organisms in aquatic ecosystems is an interesting alternative to chemical or other forms of remedification. Due to the photodynamic nature of chlorophyllin, it has the potential to control fasciolosis in developing countries. Being economically and environmentally friendly, this approach can get high public acceptance also.

Author Contributions: DC suggested the concept, design and writing the first hand manuscript. KS did extensive literature search. VKS suggested the topic and provided the technical guide. All the authors read and approved the final manuscript.

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