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A CROSS-SECTIONAL STUDY OF ETHNOPHARMACOLOGY IN THE NOAKHALI DISTRICT OF BANGLADESH AND EXPLORING POTENTIAL OCULAR IMMUNOSTIMULATORY ACTIVITY OF THE MEDICINAL PLANTS FOR THE TREATMENT OF EYE INFECTIONS

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Abstract

The authors aimed to document the medicinal plants used by the traditional healers (TH) of Noakhali district in the treatment of eye infections, as well as to include their scientific evidence relevant to the immunopharmacology towards further exploring of potential natural medicine based ocular immune system development; their question being: Do these plants have the potential for further exploration of ocular immune-modulatory activities against eye infections? And if so, is their consumption safe? This empirical ethnopharmacological study was carried out among the TH of Noakhali district, Bangladesh, with the help of a semi-structured questionnaire and the guided field walk method. In-depth information regarding medicinal plant type, preparation of medicines and parts used were obtained from the TH. Plant samples were later identified at the Bangladesh National Herbarium and a number of scientific literature were reviewed for immunostimulating activities that were frequently found in the reported plant species. The collected information indicates that the following 12 medicinal plants, *Spondias dulcis, Croton caudatus, Tagetes erecta, Ervatamia divaricata, Sesbania grandiflora, Curcuma longa, Mangifera indica, Asparagus racemosus, Centalla asiatica, Terminalia chebula, Ocimum gratissimum* and *Lawsonia inermis* are commonly used by TH to treat eye infections. A review study on several available scientific evidences attributed to the *in vivo* and *in vitro* immunomodulation properties of these species. No relevant information was found regarding the immunostimulatory activity of *Croton caudatus* and *Ervatamia divaricata*. Toxicological studies of the remaining plants (except *L. inermis, S. dulcis, E. divaricata)* were done to evaluate the safety index for human consumption. The rural inhabitants of Bangladesh mostly depend on the medicinal plants for the treatment of various bacteria, virus and fungus associated eye infections i.e., conjunctivitis, keratitis, endopthalmitis etc. Plant based Immunomodulators can become a better choice to enhance ocular immune system because of their minimal or no side effects. So, as the body of existing ethnomedicinal knowledge can be a thriving source for natural immunomodulatory bioactive compounds, it is very important to evaluate further scientific research for the exploration of new compounds that could act as ocular immunostimulatory drugs in the future treatment of eye infections.

Keywords: Ocular immunology, medicinal plants, immunomodulation, ethnomedicine, eye infections, traditional healers, ethnopharmacology, Bangladesh

Introduction

Ocular Infections are eye ailments caused by bacteria, viral, parasitic or fungal agents that occur in people of all ages. In the eye, Immunology is the basis for both health and disease. The ocular immune system protects the eye from infections and regulates the healing processes following injuries. Studies of the eye are of interest to researchers who study mucosal immunity even though the ocular surface of the conjunctiva is the only part that is covered by a mucosal surface. The study of ocular immune regulation may be a model for the understanding of immune regulation throughout the body. Moreover, the eye is considered 'immune privilege' as it does not develop the types of immune reaction that would cause bilateral damage to the organ [1]. The term immunostimulation comprises of a prophylactic or therapeutic concept which aims at the stimulation of our nonspecific immune system. This implies primarily to the non-antigen dependent stimulation of the function and efficiency of granulocytes, macrophages, complements and natural killer cells [2].

Finding healing powers in plants is an ancient concept. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists have been combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [3]. The strengthening of the internal immune system with natural products is an important concern of the African scientific biomedical research society [4]. In fact, as medicinal plants constitute an effective source of both traditional and modern medicine [5], in many areas of Africa and Asia, the use of plants with immunostimulatory properties is becoming more common. Thus it is necessary to perform pharmacological investigations, aimed at studying the effectiveness of plants used in traditional medicine and to monitor their safety indexes, allowing more efficient use [6].

A number of Indian medicinal plants have been claimed to possess immunostimulatory activity [7]. Bangladesh has the richest diversity of medicinal plants. Some medicinal plants from Bangladesh are used in the preparation of alternative medicine namely; Kobiraji, Hakimi, Unani, Ayurvedic, Homeopathic and as well as in Allopathic systems of medicine [8-9]. The rural people of Bangladesh are mainly dependent on traditional healers and they could treat various ailments like cardiovascular disorders, diabetes mellitus, schizophrenia like psychosis, snake bite, tumor, malaria, brain disorders and rheumatoid arthritis, etc. [10-17]. Various plant extracts have immunostimulatory activity as evidenced by increased proliferation of lymphocytes and production of interleukin-2 [18]. The various plant-derivatives, including alkaloids, quinones, terpenoids, phenol carboxylic acids, and high-molecular mass-compounds such as polysaccharides and glycoprotein's natural products have immunostimulatory activity [2]. Medicinal plants thus serve as therapeutic alternatives and safer choices and a large number of these plants and their isolated constituent have shown beneficial therapeutic effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory effects [19].

In our present study, we have undertaken the documentation of the application of medicinal plants, that are used by the traditional healers in Noakhali district, Bangladesh in the treatment of ocular infections, as well as the review of scientific evidences of variously reported immunostimulatory activities of these plant species, so that they can be evaluated for further scientific studies to discover new compound of drugs for developing the ocular immunostimulatory system against eye infections.

Methods

For this ethnopharmacological study, survey was conducted in Baikunthopur and Ulupara village of Noakhali district, Bangladesh. The district has an area of 3601 km^2 with a population of 3,108,083 and is subdivided into nine Upazilas. Our selected study areas, Baikunthopur and Ulupara village is covered by Chatkhil Upazila and Begumganj Upazila respectively.

The study area for this specific ethnomedicinal survey was selected by the authors themselves regarding information on the noted traditional medicinal plant practices. We are conducting systematic ethnomedicinal survey in Bangladesh since 2011 so as to know how and why medicinal plants are suggested by the traditional healers (TH), that can lead the way towards a better linking of traditional knowledge with biomedical science [20] and how the TH of Bangladesh select medicinal plants for boosting up the immune system of human body. Moreover, Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immunostimulating agents and

immunosuppressing agents have their own standing and the search for better agents exerting these activities becoming the field of interest all over the world [21].

Interviews were conducted among the TH in the Bangla language with the help of a semi structured questionnaire and guided open filed walk method. Interview protocols and field observations all followed standard ethnobotanical methods [22-26]. In this method, The TH took the authors to the areas where they collected their medicinal plants, pointed out the plants and mentioned their name and mode of use. Prior informed consent was obtained conducting interviews and researchers adhered to the ethical guidelines of International Society of Ethnobiology [27] and the final work was then submitted to and approved by the Institutional Ethnomedicine Ethical committee of TechB Herbal Solution, Bheramara, Kushita, Bangladesh. At the end of each interview plant specimens shown by the TH were collected, dried and later brought back to the Dhaka for identification at the Bangladesh National Herbarium and all the voucher specimens were deposited there.

Nomenclature of the identified species were collected from the plant list database [28]. Later meanwhile research articles, books and relevant web pages were also studied to accumulate data of immunostimulating activity of the plant species those were used by our respected traditional healers in the treatment of eye infections.

Results

The name of a total of 12 plant species were obtained from the traditional healers of Noakhali district, Bangladesh for the treatment of eye infections. The 12 plant specimens belonged to 11 families. The family Anacardiaceae (2 species) represents the highest number of species. Medicinal plant type, preparation of medicines and parts used were obtained from the TH are shown in the following table 1. And the chemical composition of each plant is given in the table 2.

Sometimes whole plants and in many cases different parts of the plants including leaf, root, stem, flower, rhizome, seed and fruit are used as medicinal components. Different preparation methods are used for administrating the medicinal plants, among them paste is the most used form, other forms are juice and infusion made by the TH for the management of eye care. Generally, Paste made from the rhizome powder of *Curuma longa* is applied to the eye. Infusion of fruits powder of *Terminalia chebula* is used as an eyewash and the TH said it is very effective for conjunctivitis. Another very effective plant name mentioned by the TH for the treatment of allergic conjunctivitis, was *Tagetes erecta*. Juice obtained from crushed leaves, stems and flower of *T. erecta* were mixed together and applied to eyes. Rest of the preparation methods are shown in the table 1.

Different research articles, web pages and books report that the plant species used by the traditional healers in the treatment of eye infections, has validated *in vivo* and *in vitro* immunostimulatory activities, which indicates that these plant species can be potential for further scientific evaluation of ocular immune stimulating activities. When such studies have been conducted, the potential toxicity of each plant is described. In the following section we have discussed more regarding this topic.

Discussion

Pathophysiology of Eye Infections

Healthy corneas are avascular structures, so the normal host-defense mechanism also includes, physical barriers (eyelids, corneal epithelium), tear film turnover, Langerhans cells, Immunoglobulins (esp., IgA) and other soluble macromolecules (mucin, lysozyme, lactoferrin, lipocalin, lacritin, β-lysine) that are present in the tear film; and it is associated with both T-cell and B-cell (antibody) mediated pathways [29]. Once infection occurs, the inflammatory cells (Neutrophils, Plasma cells, Mast cells, Eosinophils) and mediators (Cytokines (ILs), TNF, Defensins) [30] from the tear film, limbal vasculature and anterior chamber, enter into the corneal stroma, facilitated by corneal neovascularization. They surround the beginning ulcer to create a Hypopyon, a layered deposit of inflammatory cells and immune complexes in the anterior chamber. While the inflammatory response may help to fight infection, the resulting activation of the complement pathways and the release of tissue-destructive enzymes (elastase, collagenases, proteases, coagulases, lipases, fibrinolysins) and bacterial exotoxins and endotoxins* contribute to the destruction of the peripheral corneal stroma, thus causing swelling, necrosis, scarring and thinning of the stromal lamellae that leads to decreased vision in the long term and, in severe cases, perforation of the globe in the short term; typically, the neighboring tissues are inflamed as well [29, 31].

[*Endotoxins are responsible for corneal ring infiltrates, which are polymorphonuclear cells in stroma attracted by C-pathway and properdin-activated chemotoxins. Ring infiltrates are believed to be antigen-antibody precipitates and

are also seen in viral, fungal and acanthamoeba infections.]

Ocular Defense Mechanism to Infection

The ocular surface is constantly exposed to a wide array of microorganisms. The ability of the outer ocular system to recognize pathogens as foreign and eliminate them is critical to preserve corneal transparency. A combination of anatomical and immunological defense mechanisms has evolved to protect the outer eye. These host defense mechanisms are classified as either a native nonspecific defense or a specifically acquired immunological defense requiring previous exposure to a particular antigen for the development of acquired immunity. Sight threatening immunopathology with autologous cell damage also can take place after these reactions [32].

The defense of the ocular surfaces presents a unique challenge to maintain integrity against microbial, inflammatory and physical assault, while at the same time minimizing the risk of loss of corneal transparency [33]. The vascular supply to the surface of the eye is a major conduit of the immune defenses. The ocular inflammatory response involves vascular dilation and exudation of immunologically active substances and cells, including macrophages, polymorphonuclear leukocytes, and lymphocytes [34]. The tear film contributes to the neutralization of toxic substances and is comprised of three layers: oil, aqueous, Mucous. Tear flow mechanically bathes the anterior surface of the eye, preventing the adherence of microorganisms, and flushes allergens and foreign particles into the lacrimal excretory system. The mucous layer of the tear film entraps foreign material, which facilitates its removal [35]. The tear film also contains several immunologically active substances that participate in both general and specific ocular defense [36]. The conjunctiva associated lymphoid tissue is sub-epithelial tissue packed with B and T lymphocytes. B-cell precursors mature when exposed to local antigen, which then proceed to the regional lymph nodes where they transform into plasma cells, which return via the bloodstream to the conjunctiva to produce their specific antibodies (e.g. IgA) [36].

Similarly, T-cell precursors are locally sensitized, travel to regional nodes, and then hematogenously return to the conjunctiva to provide cellular defense [37]. The Langerhans cells possess receptors for immunoglobulins, complement and antigen. They recognize, phagocytize and process certain antigens for the epithelial surface stroma [34]. Langerhans cells stimulate helper T and B cells that collaborate with other lymphocytes (killer, suppressor T cells) to enlist a strong cellular and humoral immune response. During inflammation Langerhans cells migrate toward the center of the cornea and may participate in the secretion or release of inflammatory mediator substances [38]. Polymorphonuclear leukocytes possess the ability to ingest and kill microorganisms by two main pathways; the oxygen-dependent pathway is based on postphagocytic intracellular production of oxygen radicals (oxidants) and the oxygen-independent pathway is based mainly on the function of antimicrobial proteins called defensins. Defensins are peptides that possess broad-spectrum antimicrobial activity in vitro, killing a variety of gram-positive and gram-negative bacteria, some fungi and a wide range of ocular pathogens [34, 39].

Immunopharmacological Activities of the Plants

Globally, many bioactive compounds or molecules are being screened for their ability to modulate inflammation and immune functions. Although plants are a rich source of bioactive molecules, more than 90 % are yet to be screened for biological activity. The use of ethnomedical information has contributed to healthcare worldwide, even though efforts to use it have been sporadic. There are 122 compounds obtained from only 94 plant species, that are used globally as drugs and 80% of these have had an ethnomedical use [40]. For this present study, available scientific literature was screened for reports that may validate the use of the surveyed immunostimulatory plants that were utilized by the traditional healers. Immunostimulating property of *Asparagus racemosus* has been shown to protect the rats and mice against experiment-induced abdominal sepsis [41]. Dhuley has reported the revival of macrophage chemotaxis and reduction of IL-1 and tumor necrosis factor (TNF)- α by the oral administration of *A. racemosus* root extract in an ochratoxin-treated mouse model and alcoholic extract has been found to enhance both humoral and cell mediated immunity of albino mice injected with SRBCs as particulate antigen [42]. A study revealed that mixed Th1/Th2 activity of *A. racemosus supports* its immunoadjuvant potential [43]. Oral administration of decoction of powdered root of the plant has been reported to produce leucocytes and predominant neutrophilia along with enhanced phagocytic activity of the macrophages and polymorphs. Percentage mortality of *A. racemosus* treated animals was found to be significantly reduced while survival rate was comparable to that

of metronidazole and gentamicin [44]. Extracts and formulations prepared from exhibited various immunopharmacological actions such as an increase in white cell counts, haemagglutinating and haemolytic antibody titres in cyclophosphamide (CP)-treated mouse ascitic sarcoma [45]. Therefore, *A. racemosus* is a potent immunostimulant [46-47] that also has significant stimulatory effects on insulin secretion mediated through physiological pathways [48].

The immunostimulant properties of *Centella asiatica* have been reported to be comparable to recombinant interferon α-2b injection [49]. Authors have stated its immunomodulating activity to be due to non-specific cellular and humoral immune responses. The data available till date, suggest that it may have chemopreventive or anticancer potential [50]. It was seen that ethanolic extract of *C. asiatica* could combat immunosuppressive effect of cyclophosphamide ($P < 0.01$). The study [51] suggests that *C. asiatica* can be regarded as biological response modifiers and can be utilized for the development of immunostimulating agent among plant sources. Hence this plant is said to have played a role in the activation of T-lymphocytes and the augmentation of circulating antibodies [51] and the reticulo-endothelial stimulating activity of the alcoholic extract of *C. asiatica* have also been reported [52]. Pectin isolated from *C. asiatica* showed immunostimulating activities [53] and triterpenoid, saponins [54] and methanol extracts showed preliminary immunomodulatory effect [55].

Curcumin present in *Curcuma longa* has been suggested to modulate the proliferation and cellular response of various immune cell types, such as T cells, B cells, macrophages, neutrophils, NK cells and dendritic cells [56, 57]. Recent findings also suggested that aromatic turmerone and Polysaccharides isolated from *C. longa* possess immunostimulating activities in human peripheral blood mononuclear cells [58, 59].

Methanol extract of *Lawsonia inermis* leaves at 1 mg/ml concentration have displayed immunostimulant action as indicated by promotion of T-lymphocyte proliferative responses. Seven compounds were isolated adopting the lymphocyte transformation assay (LTA)-guided fractionation of the total methanolic extract of henna leaves [60]. Naphthoquinone fraction obtained from *L. inermis* leaves showed significant immunomodulatory effect [61]. *Mangifera indica* (Mango) is known as the king of fruits and is a good source of Vitamin C, Vitamin E and Vitamin B6 which provides immunity and helps to develop resistance against infections. Essential oils from peels of *M. indica* cultivar zebdeya and *M. indica* cultivar cobaneya showed appreciable non-specific immunostimulant activity (low Macrophage migration index) [62]. The Alcoholic extract of the stem bark containing mangiferin had promising *in vivo* immunostimulatory effect. It was confirmed that the immunostimulatory effect was due to cellular and humoral antibody-mediated activation of T and B cells [63]. The study showed that Aqueous ethanolic extract of *M. indica* stem bark may increase the rate of erythropoiesis, slow down the natural process of oxidative breakdown of RBCs and may also promote immune-stimulatory activities of WBC [64]. The significant increase in WBC and the differential leukocytes counts in the test animals suggests that the extract may have immunological properties, by stimulating increased production of WBCs, thereby boosting the defence system of the animals. This is in agreement with earlier findings [65] that *M. indica* extract might have immunostimulating properties, which may account for the use of the plant for medicinal purposes by traditional medical practitioners. Herbal extracts have potential application as immunostimulants and could act against a broad spectrum of pathogenic microorganisms. The effect is dose dependent and there is the tendency for overdose to occur, therefore dosage optimization is strongly recommended [66].

The methanolic extract of *Ocimum gratissimum* has significantly increased the intercellular reduction of NBT (Nitro Blue Tetrazolium) dye to formazen (deep blue compound) by the neutrophils, confirming the intracellular killing property of phagocytosing neutrophils. From the results obtained [67], it can be concluded that the methanolic extract of *Ocimum gratissimum* leaves stimulates cell-mediated immune system by increasing neutrophil function, phagocytic activity.

The kinetic study of bactericidal activities revealed that the butanol fractionated extract of the stem bark was effective against Gram negative bacteria. This study suggests that the stem bark of *Sesbania grandiflora* contains promising antibacterial substances for clinical purposes [68]. Ophthalmic drug delivery system is extremely interesting and a highly challenging endeavors [69, 70]. A study of *in situ* gel of the *Sesbania grandiflora* flower extract showed active against the microorganisms *P. aeruginosa, S. aureus, E. coli*, and fungus *C. albicans*, all of which cause the bacterial conjunctivitis and *S grandiflora* could be a promising in situ gelling formulation for long acting ocular delivery [71].

External immunostimulants are very helpful to induce immune responses against microbes to prevent infections in immunocompromised conditions [72]. A preliminary study findings suggest that methanolic extract of fruits of *Spondias dulcis* caused stimulation of B cells for IgM production and hence it may be useful for enhancing the immunity of the body [73].

Tagetes erecta contains carotenoid and a study revealed that carotenoid treatment elevated cell-mediated immunity (as measured by the PHA skin test) [74]. The cells of the immune system are particularly sensitive to oxidative stress and may benefit substantially from the free radical–trapping ability of carotenoids, as the immune response itself produces reactive oxygen species that disrupt the intercellular signals sent via lipid-rich, membrane-bound receptors [75]. An enormous body of literature has accumulated over the past 20 years demonstrating the potency of carotenoids as antioxidants and immunostimulants in humans and other mammals [76, 77] and in nature, carotenoids have been implicated in immunostimulation [78].

Terminalia chebula is a plant widely used in the traditional medicinal systems of India. The aqueous fruit extract of *T. chebula* has been investigated for its effect on cell-mediated and humoral components of the immune system in mice. Administration of *T. chebula* extract produced an increase in humoral antibody (HA) titer and delayed-type hypersensitivity (DTH) in mice. It was concluded that the *T chebula* extract is a promising drug with immunostimulant properties [79]. The study [80] confirms the immunomodulatory activity of ripe *T. chebula* fruits as evidenced by increase in the concentration of antioxidant enzymes, GSH, T and B cells, the proliferation of which play important roles in immunity. This phenomenon also enhances the concentration of melatonin in pineal gland as well as the levels of cytokines, such as IL-2, IL-10 and TNF-α, which play important roles in immunity**.**

Toxicity of the Plant Species

Most of the toxicological studies report that toxic effects due to the use of herbal medicine are mostly associated with hepatotoxicity. Other toxic effects are on the kidney, nervous system, blood and cardiovascular system and it has been published in medical journals that mutagenecity and carcinogenicity may also occur [81]. Alcoholic extract of the root of *A. racemosus* has been shown to significantly reduce the enhanced levels of alanine transaminase, aspartate transaminase and alkaline phosphate in CCl_4 induced heptic damage in rats [82, 83], indicating an antihepatotoxic potential of *A. racemosus*. Systemic administration of higher doses of all its extracts did not produce any abnormality in the behavior pattern of mice and rat [84]. LD_{50} of the product Lactare has not been assessed since it did not produce mortality even up to oral dosage of 64 g/kg [85] and rather the roots of *A. racemosus* showed diuretic activity at a dose of 3200 mg/kg without signs of acute toxicity [86]. A study demonstrated that *Centella asiatica* seemed to be destitute of toxic effects, which could compromise the medicinal use of this plant in folk medicine [87]. The extract was also shown to exert low toxicity by chronic application with the LD_{50} value of 675 mg/kg [88]. The result of the study [89] suggest that *Curcuma longa* and *Curcumin* can be used as dietary supplement in order to prevent aflatoxin B_1 induced toxicity by modulating the extent of lipid peroxidation and augmenting the antioxidant defense system. The *C. longa* extract has multiple therapeutic activities that block the cardiac, hepatic, and renal toxicities induced by Doxorubicin, and it also possibly acts as a free radical scavenger [90].

Acute toxicity of *Lawsonia inermis* extracts were checked *in vivo* in mice. No mortality was observed during the study and all the signs of toxidrome were negative [91]. From the literature [81] it has been revealed that *L. inermis* exhibited significant hepatoprotective, antioxidant, antiinflammatory, antibacterial, analgesic and adaptogenic effects indicating that it is a safe substance to be used as a drug ordinarily.

The lethal dose (LD_{50}) of the aqueous ethanolic extract of *Mangifera indica* stem bark has been estimated as > 5000, suggesting that the extract may have low toxicity [64]. Another study has found that oral or dermal administration, the extract of *M. indica* showed no lethality at the doses of 2,000 mg/kg body weight and no adverse effects were found [92]. Therefore it was earlier established that any substance with LD_{50} estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans [93, 94]. According to the study [95], the oil of *Ocimum gratissimum* can be considered safe for humans. From the serum biochemistry result, *O. gratissimum* extract appears to be safe at low and moderate doses of 400 and 800 mg/kg, but some mild toxic changes were elicited in the liver and kidney of rats treated with the 1600mg/kg dose of the extract for 28days [96]. Another study concluded that ethnomedicinal application of *O. gratissimum* is quite safe at lower doses but it is hepatotoxic and nephrotoxic at higher

doses [97]. Petroleum ether extract of *Sesbania grandiflora* fruits possesses significant hepatoprotective activity ($p<0.001$) at a high dose of 400mg/kg and thus supports the traditional application of the same under the light of modern science [98]. The results of the another study revealed that Oral administration of an ethanolic extract of *S. grandiflora* leaves (200 mg/kg/day) for 15 days produced significant hepatoprotection against erythromycin estolate (800 mg/kg/day)-induced hepatotoxicity in rats, the effect of *sesbania* being compared with that of silymarin, a reference hepatoprotective drug [99].

The results of the study [100] suggest that the chloroform fraction is not acutely toxic to the rats thereby providing a support to the use of *Tagetes erecta* flower in indigenous system of medicine. Treatment of chloroform fraction at 200 and 400 mg/kg doses did not make any significant alterations on the hematological and biochemical parameters of rats when data were compared with that of untreated controls. Histopathological examination also showed no detectable changes in liver, kidney, heart and lung of chloroform fraction treated rats. This study revealed that the chloroform fraction of *T. erecta* had no toxic effects [100]. Moreover, it was found that Phytoconstituents such as flavonoids, terpenoids and steroids of *T. erecta* were responsible for the observed hepatoprotective activity [101].

In the oral toxicity study of *Terminalia chebula*, the single oral dose of the acetate (EtOAc)-soluble portion of a *T. chebula* ethanol extract at 2000 mg/kg did not produce mortality or abnormal lesions in the internal organs of rats. The results of a 14-day orally repeated dose showed that the EtOAc-soluble portion of *T. chebula* ethanol extracts gave no adverse effects at dosages of 2000 mg/kg in rats in the study [102]. From another study, the water extract of *T. chebula* given orally to female and male rats did not produce either acute or chronic toxicities in rats [103]. After comparing the immonopharmacological actions with the toxic effects of these plants, it was observed that these plants not only showed immunostimulatory and immunomodulatory properties by enhancing the cellular and humoral immunities, but also each of them had some individual properties that differentiated them from each other. For instances , Hepatoprotective property was shown by *A. racemosus, L. inermis, S. grandiflora* and *T.erecta;* Antioxidant properties were seen in *C. longa, T. chebula, T. erecta, L. inermis*; *C. longa & T. erecta* prevented free radical toxicity; *C. asiatica* was chemopreventive & combated immunosuppression; *S. dulcis* increased humoral immunity in immunocompromised people; *C. longa* also blocked cardiac and renal toxicities; *A. racemosus* acted as a diuretic & stimulated insulin secretion*; S. grandiflora* had an *in situ* gel form for extended night use; *M. indica* contained vitamins, B_6, C, E, and maintained RBC level by increasing erythropoietin level and decreased RBC breakdown; etc.

The toxicological study of *C. asiatica, S. grandiflora, A. racemosus, T. chebula* revealed low toxicity and high LD_{50} levels indicating a high safety index with minimal adverse effects, even when administered in high doses. But *T. erecta* and *O. gratissimum* were found to be safer in low to moderate doses and toxic in high doses. *M. indica* although having a low toxicity and high LD_{50} level, was seen to be dose dependent thus with a risk of overdosing.

Conclusions

This preliminary study reports that extracts and isolated compounds of the 12 plants used by the traditional healers contain phytochemicals ie., alkaloids, flavonoids, tannins, terpenoids, saponins, glycosides etc., with immunostimulatory activities, that promote both cellular and humoral immunities, as evidenced by result of this survey. 5 of these plants have high safety indexes whereas 2 plants have moderate safety index, as revealed by the toxicological studies. Our study could be used to guide pharmacological and therapeutic evaluation by research on these plants at a molecular level and all these studies are backed up by supporting reports obtained from various research papers, books and websites, thus to an extent answering the questions asked in the introduction of this paper and supporting the use of Bangladeshi medicinal plants for treating eye infections and claiming them to be safe. Moreover, since Bangladesh is a rich and thriving source of different natural medicinal plants, research could be undertaken for the exploration of newer bioactive compounds from other plants and to safely use these plant-based immunomodulatory drugs to improve the ocular immune system and for the future treatment of ocular infections as plant-based drugs have minimal or no side effects in comparison to that of synthetic drugs.

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Conflict of interest

The Authors declare no conflict of interest.

Authors' Contribution

MN Ahmed has made the conception and design of the manuscript, MNK Azam and MN Ahmed conduct the survey among the TH in Noakhali district, SB has been involve in drafting the manuscript, finally all the authors read and edited the manuscript for final submission.

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Table 1 : Medicinal Plants and their formulations used by the traditional healers of Noakhali district, Bangladesh in the treatment of Eye Infections.

SN	Botanical Name	Family	Vernacular/English Name	Part (s) used	Formulations
1	*Asparagus racemosus* Willd.	Liliaceae	Shotomuli/ Asparagus	Root	Roots are crushed with water to make paste, then applied to the eye
2	*Centella asiatica* (L.) Urb.	Apiaceae	Thankuchi/Pennywort	Whole plant, leaf	Juice obtained from crushed leaves or whole plant is applied to eye
3	*Croton caudatus* Geiseler	Euphorbiaceae	Goinnahari/Caudated croton	Leaf	Infusion of leaves or sap from the leaves is applied to the eye
4	*Curcuma longa* L.	Zingiberaceae	Holud/Turmeric	Rhizome	Paste made from rhizome powder applied to the eye
5	*Ervatamia divaricata* (L.) Burkill	Apocynaceae	Togor/Burkill	Flower	Juice from flower is applied to eye
6	*Lawsonia inermis* L.	Lythraceae	Mehedi/Henna	Leaves	Paste of leaves is applied to the eye
7	*Mangifera indica* L.	Anacardiaceae	Aam/Mango tree	Seed	Crushed seeds used to make paste and applied to the eye.
8	*Ocimum gratissimum* L.	Lamiaceae	Tulshi/African Basil	Leaf	A piece of cloth is soaked with juice obtained from crushed leaves is tied to the eye area
9	*Sesbania grandiflora* (L.) Pers.	Fabaceae	Bokful/Swamp pea	Flower	Juice of flower put in the eyes as an eyewash
10	*Spondias dulcis* Parkinson	Anacardiaceae	Amra/ Ambarella	Leaf, fruit	Paste of crushed fruits and leaves mixed and is applied to the eye
11	*Tagetes erecta* L.	Asteraceae	Genda/African Marigold	Leaf, stem, flower	Juice obtained from crushed leaves, stems and flower is applied to eyes
12	*Terminalia chebula* Retz.	Combretaceae	Horitoki/ Chebulic myrobalan	Fruit	Infusion of fruit powder is used as an eyewash

Table 2: Reported phytochemical compounds of each medicinal plants used by the traditional healers of Noakhali district, Bangladesh

SN	Botanical Name	Phytochemical compounds	Reference
1	*Asparagus racemosus*	Saponins (Shatavarins I-IV), Immunoside, Aspargamine A, Racemofuran, dihydrophenantherene, carbohydrates, glycosides, mucilages	104, 105
2	*Centella asiatica*	Triterpenoids (Asiatic acid, cenellic acid, brahminoside, thankuniside), Asiaticosides, polyacetylenes, Tannins, Saponins, sesquiterpenes, Flavonoids, Amino acidm, Essential oils, Reducing sugar	106, 107
3	*Croton caudatus*	Triterpenoids (taraxerone, taraxerol and taraxeryl acetate), Tannins, Saponins, flavonoids, cyanogenetic glycosides, alkaloids, phenols, dotriacontamol, bomyrin, b-sitosterol, Essential oil (ascaridole)	108
4	*Curcuma longa*	curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), oil-free aqueous extract (COFAE), turmerones (volatile oil), sesquiterpenoids, flavonoids, glycosides	59, 109, 110
5	*Ervatamia divaricata* (L.) Burkill	Campesterol-D-glucoside, stigmosterol, Asiatic acid, Kaemferol, 11- hydroxyl voabacristine and heyneanine.	111
6	*Lawsonia inermis*	P-coumaric acid, Lawsone, Apigenin, Luteolin, 2-methoxy-3-methyl-1,4-naphthoquinone, cosmosiin, apiin, glycosides, gums, mucilage, Carbohydrates, Flavonoids, Tannins, phenolic compounds, terpenoids, quinones, coumarins, xanthones	60, 112
7	*Mangifera indica*	protocatechic acid, catechin, mangiferin, isomangiferin, tannins, gallic acid, terpenoids, sterols, flavonoids, phlabotannins, saponins	113, 114
8	*Ocimum gratissimum*	Tannins, terpenoids, steroids, flavonoids, cardiac glycosides, phlabotannins, antraquinones, alkaloids, phenols	115, 116
9	*Sesbania grandiflora*	Alkaloids, Flavonoids, Glycosides, Tannin, Anthraquinone, Steroid, Pholobatannins, Terpenoids	117, 118
10	*Spondias dulcis*	Alkaloids, Flavonoids, Steroids, Terpenoids, saponins, tannins, cardiac glycosides	119
11	*Tagetes erecta*	Flavonoids, Steroids, Triterpenoids, saponins, tannins, phenols, alkaloids, glycosides	120, 121
12	*Terminalia chebula*	Triterpenoids, tannins, phenols, chebulic acid, glycosides, sugar, steroids, phosphoric acid.	122