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Probable Approach of Safe Mass Sanitization

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Abstract

Currently, the Novel Coronavirus, also known as SARS-CoV-2, have 3,49,47,761 confirmed cases, including 980 031 deaths till 4th October 2020 globally, reported by World Health Organization. The virus is transmitted from human to human but it can also transmitted from a surface to human. An advisory given by Ministry of Health & Family Welfare (Govt. of India) that Spraying of individuals or groups with chemical disinfectants is **NOT recommended** under any circumstances. Chemical sanitization leads to potentially gastrointestinal effects, bronchospasm, irritation of eyes and mucous membrane, as a whole severe health issues. There is an urgent necessity to find a safe mass sanitization technique that may slow down the spread of virus. The aim of this study is to develop a herbal mass-sanitization formulation prepared from neem bark extract (*Azadirachta Indica*) as potential inhibitors for COVID-19 Main Protease. A sensor based Mass Sanitization Sprinkler is an innovative technique which can sanitize people within 30 seconds in a crowed area. It is very cost effective, uses safe and effective herbal sanitization formulation. Anti-microbial / viral screening of this formulation has been done and tested in laboratory. This would be an effective approach as a biosafety system which will act as a multi-barrier concept and maintain virus free campus. Hence this system promotes healthy hygiene and provides protection for future.

Keywords
Disinfectants, Neem, COVID-19, Sprinkler, Biosafety, Mass-sanitization.

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INTRODUCTION:

Coronavirus disease (COVID-19) is an outbreak situation, which is declared to be a pandemic by the World Health Organization (WHO) [1]. This is a severely infectious disease caused by a newly discovered coronavirus, which have a high human-to-human transmissibility as well as it can also be transmitted from surface to human. Corona virus was first initiated on 31st December 2019, when a certain case in humans having pneumonia unknown etiology emerged in Wuhan, China [2]. Currently, the total number of COVID-19 cases has been raised to 3,49,47,761 confirmed cases with 10,33,998 deaths as well till 25th September 2020 [3]. The virus is mainly transmitted through droplets generated when an infected person coughs, sneezes, or exhales. These droplets are too heavy to hang in the air, and quickly fall on floors or surfaces and can be transmitted by breathing in the virus if someone is within close proximity of an infected person, or by touching a contaminated area[4].

Today, due to this pandemic, proper sanitization and maintaining physical distancing is more important than anything else. But in country like India where maintaining social distance is a real challenge, so a sanitization process like this can be a potential multi

barrier preventive approach. Due to germs and viruses, there can be several medical emergencies that can turned out to be an unprecedented world health dilemma to the entire world, wherein hygiene is the key defence against such crisis.

Hence the term 'sanitization' has earned much attention. This could be sanitization of hands or sanitization of surface tops or sanitization of mass around us that people are getting in contact throughout the day. It is one of the most effective step against viruses to control their spread and break the chain.

There are different types of sanitization processes that are available in the market. Some of the popular sanitization methods are:

- Antimicrobial sanitization
- Foam cleaning sanitization
- Chemical sanitization
- Thermal sanitization that is done with the use of either steam or hot water
- Pressure cleaning sanitization is done with the help of a strong force of flowing water.

The type of sanitization method chosen depends on the nature of the surface that needs to be cleaned and the environment where the surface is situated. The process of sanitization can be done with the use of a large number of products and chemicals. Some of the most common chemicals in this context are Chlorine-based cleaners, Quaternary ammonium compounds, 80% Alcohol-based cleaners, Hydrogen Peroxide etc [5].

Chemical disinfectants are recommended for cleaning and disinfection only of frequently touched areas/surfaces by those who are suspected or confirmed to have COVID-19 but still there is a finding going on for safe mass sanitization formulation that can be sprayed over the human body for disinfection. Chemical sanitization has lot of drawback over advantages. Spraying a chemical disinfectants to an individual or group is physically and psychologically harmful. Spraying chlorinated solution on individual can lead to irritation of eyes, skin and potentially causes the gastrointestinal effects such as nausea and vomiting. Inhalation of sodium hypochlorite can lead to irritation of mucous membranes to the nose, throat, and respiratory tract and may also cause bronchospasm, stated by Heath Ministry of India. Also these chemicals are reported to be corrosive which causes damage to the objects and the property as well due to regular use. Obnoxious smell is a continuous problem with chemical formulation. Though, alcohol is safer for mass sanitization but market price of alcohol is very much high as compared to other chemicals and availability is also is serious issue. Due to these adverse reactions and complications, an advisory given by Ministry of Health & Family Welfare that Spraying of individuals or groups with chemical disinfectants is **NOT recommended** under any circumstances.

The world health organization estimates that 80% of the population living in the developing countries relies exclusively on traditional medicine for their primary health care. The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The bark of neem plant *[Azardirachata indica Linn* (Meliaceae)] has been widely used as a traditional medicine for many centuries in tropical countries. Earlier studies have confirmed that neem bark extract contains some substance with strong anti-inflammatory, anti-viral, anti-oxidant and many more properties [6, 7]. An In-vitro antiviral activity of neem bark extract was studied against herpes simplex virus type-1 infection which was found to be very much efficacious with no side effects [8].

In this present study, the central aim was to develop a herbal mass sanitization formulation prepared from neem bark extract (*Azadirachta Indica*) as potential inhibitors for COVID-19 Main Protease.

Need of mass Sanitization:

Today, during this pandemic situation, corona worriers and emergency health workers are having the high risk of getting contamination from this dangerous virus. Day by day, these worriers are getting infected in a rampant speed. As India is in unlock phase, the risk of contamination is now has become increased and the situation is getting worsen. India is also planning to restart the schedule wise regular railway, metro, international airline services. Schools and colleges are still continuing in online mode from last 6 months and students are facing a lot of difficulties during this pandemic. Railway and metro station, airports, market places, educational institutions, offices etc. are the over-crowded places where there is a lot of gathering happens every day. The aim of the mass sanitization is to control the infection and maintaining a proper hygiene in crowded areas like different societies, colleges, schools, hospitals, offices, banks, malls etc., where there is a large gathering and high chance of transmission of any infection.

A safe mass sanitization formulation can be developed, then the risk of getting contamination from infected surface is reduced and minimized. A unique mass sanitization sprinkler can sprinkle the formulation over the human body and the other objects being carried by. Advanced mass sanitization

sprinkler can be installed in entrance and exit of these areas to sanitize a large number of mass in limited time. This sprinkler system should be installed at such area where a huge mass of people can gather. The purpose of using herbal formulation is to spray over human body to disinfect them with no adverse effects. This might be an effective biosafety system which can act as a multi-barrier approach and maintain virus free campus.

Advanced Mass-Sanitization Sprinkler:

This unique Advanced Mass Sanitization Sprinkler is a sensor based sanitization machine which can use the technique of eco-friendly fogging disinfection system. The machine can be installed on a plane platform in entrance and exit of these areas to sanitize a large number of mass in a limited time-period. A concentrated sanitizing solution will be atomized into a non-flammable; penetrating, dry mist, which can kills pathogens efficiently. A stage will be prepared at the centre of the base of the machine. When a person will stand on the stage, the sensor will get on and the sprinkler in the centre of the machine spray the sanitizer on the person for 30 seconds. After that the sprinkler will automatically turned off. This sprinkler machine can also be operated manually, as it has both options for sensor based or manual operation. It contains a portable chemical reservoir to store the sanitizer solution, which will be prepared according to the need. This sprinkler can be useful for sanitizing and disinfecting an entire person while passing through it in a very short span.

How Neem can help here?:

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The world health organization estimates that 80% of the population living in the developing countries relies exclusively on traditional medicine for their primary health care. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathic system of medicine are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants [6, 7].

Neem is an omnipotent tree and a sacred gift of nature. The latinized name of neem, *Azadirachta indica* (derived from the Persian) is a member of the Meliaceae family and its role as health-promoting effect is attributed because it is rich source of antioxidant [9]. Neem ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases [10, 11]. The divine tree *Azadirachta indica* (Neem) is perhaps the most useful traditional medicinal plant. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. The tree is still regarded as **"Village Dispensary"** in India. Most of the parts of the plant such as fruits, seeds, leaves, bark and roots, which contain 140 chemically active compounds that has been isolated and are being used traditionally as a cure for many diseases. This compounds have been identified with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer, anti-carcinogenic, anti-hyperglycaemic, immune-modulatory, anti-mutagenic and antifungal properties [12]

Taxonomic Position of *Azadirachta indica*:

Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	Azadirachta
Species	Indica

Active Chemical Constituents of Neem:

Azadirachta indica L. (Neem) shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, quercetin, polyphenolic flavonoids, and ascorbic acid. Because of their special property of possessing multiple ingredients, they show multifarious therapeutic actions [13-14].

Neem elements are mainly divided in two groups: Nonisoprenoids and Isoprenoids. The non-isoprenoids comprise of proteins, sulphurous compounds, carbohydrates and polyphenolics including dihydrochalcone, flavonoids, coumarin,

and aliphatic compounds. The isoprenoids consist of di-terpenoids and tri-terpenoids which include azadirone, protomeliacins, limonoids and some derivatives such as nimbin, vilasinin, salanin and azadirachtin. By an alcoholic extract of neem leaves a dose dependent substantial decrease in blood pressure has also been reported [15].

Anti-oxidant Activity:

Free radical or reactive oxygen species are one of the main culprits in the genesis of various diseases. However, neutralization of free radical activity is one of the important steps in the diseases prevention. Antioxidants stabilize/deactivate free radicals, often before they attack targets in biological cells [16] and also play role in the activation of anti-oxidative enzyme that plays role in the control of damage caused by free radicals/reactive oxygen species. Medicinal plants have been reported to have antioxidant activity [17]. Plants fruits, seeds, oil, leaves, bark, and roots show an important role in diseases prevention due to the rich source of antioxidant. Neem bark extracts have been studied for their antioxidant activity and results of the study clearly indicated that all the tested bark extracts/fractions of neem grown in the foothills have significant antioxidant properties [18].

The results of the study also revealed that root bark extract exhibited higher free radical scavenging effect with 50% scavenging activity at 27.3 μg/mL and total antioxidant activity of this extract was found to be 0.58 mM of standard ascorbic acid [19]. Other results of study concluded that tested bark extracts/fractions of neem grown in the foothills (subtropical region) have significant antioxidant properties [18].

Anti-viral activity:

A study done by M. B. Yerima in 2012, showed that neem bark (NBE) extract significantly blocked HSV-1 entry into cells at concentrations ranging from 50 to 100 μg/mL [20]. Furthermore, blocking activity of NBE was noticed when the extract was pre-incubated with the virus but not with the target cells suggesting a direct anti-HSV-1 property of the neem bark [21].

Tiwari et al. showed the in vitro antiviral activity of neem (A. indica L.) bark extract against herpes simplex virus type-1 infection [22]

Skin diseases:

Neem has a remarkable effect on chronic skin conditions. Acne, psoriasis, eczema, ringworm and even stubborn warts are among the conditions that can clear up easily when high quality, organic neem oil is used. Neem has been used in Siddha medicine for the treatment of skin diseases [23]. In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin.

Anti-inflammatory Activity:

A study done by Alam et al. showed the anti-inflammatory activity of epoxy-azadiradione against macrophage migration inhibitory factor [24]. Another study done by Thoh et al. found that azadirachtin interacts with retinoic acid receptors and inhibits retinoic acid-mediated biological responses [25].

Safety, Toxicities, and LD_{50} Values of Neem:

The measurement of toxicities of natural compound is crucial before their application in health management. Various studies based on animal model and clinical trials confirmed the neem is safe at certain dose and on the other side neem and its ingredients showed toxic/adverse effect. Several studies reported, in children, neem oil poisoning causing vomiting, hepatic toxicity, metabolic acidosis, and encephalopathy [26-28] and another study based on rat model showed that administration of leaf sap caused an antianxiety effect at low doses, whereas high doses did not show such types of effect [29]. An important study based on rats model showed that azadirachtin did not show toxicity even at 5 g/kg bw [30]. A study based on rabbit was performed to check the toxicological analysis and results of the study showed there was progressive increase in body weight in both the test and control animals, and during the entire duration of the administration of the neem extract, there was no observed sign of toxicity in both groups [31].

A study reported that lethal median doses (LD50) recorded for neem stem bark extracts were 31.62 and 489.90 mg/kg body weight, respectively [32]. The LD50 of water extract of leaves and seed of A. indica were 6.2, 9.4 mL kg−1, respectively [33]. Lethal dose values were calculated with probit analysis and LD50 and LD90 values were found to be 8.4 and 169.8 μg/fly of neem extract, respectively [34]. A test for acute oral toxicity in mice revealed that LD50 value of approximately 13 g/kg body weight [35].

Herbal Sanitization over Chemical Sanitization:

The world is facing a pandemic in the form of the COVID-19 outbreak. In this situation, one cannot be sure about the authenticity of information coming their way. And in today's time where information exchange is rampant, it can lead to a ton of questions about which method to use for disinfection and sanitization against COVID-19. The only thing that can assure the masses in these grave times is education towards the prevention of COVID-19.

During this period, many hospitals, police stations, banks, certain districts and local bodies have installed mass sanitization machine where chemical

disinfectant formulations were used and it gained a lot of media attraction. The Purpose of using disinfectants as spray over human body to disinfect them from COVID-19 to maintain a virus free campus.

Chemical sanitization is achieved through the use of chemical compounds capable of destroying disease causing viruses/bacteria. Common chemical sanitizers are chlorine (bleach), iodine, and quaternary ammonium. Chemical sanitization is performed in two ways; by full immersion or rinsing, swabbing or spraying. [36]

Disinfectants are chemicals that destroy disease causing pathogens or other harmful microorganisms. It refers to substances applied on inanimate objects owing to their strong chemical properties [37]. Chemical disinfectants are recommended for cleaning and disinfection only of frequently touched areas/surfaces by those who are suspected or confirmed to have COVID-19.

Ministry of Health & Family Welfare has received many queries regarding the efficacy (if any) of use disinfectants such as Sodium hypochlorite spray used over the individuals to disinfect them. After that an advisory given by Ministry of Health & Family Welfare that Spraying of individuals or groups with chemical disinfectants is **NOT recommended** under any circumstances. Spraying an individual or group with chemical disinfectants is physically and psychologically harmful. Spraying of chlorine on individuals can lead to irritation of eyes and skin and potentially gastrointestinal effects such as nausea and vomiting. Inhalation of sodium hypochlorite can lead to irritation of mucous membranes to the nose, throat, and respiratory tract and may also cause bronchospasm, stated by Heath Ministry of India.

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Neem is an omnipotent tree and a sacred gift of nature. The tree is still regarded as **"Village Dispensary"** in India. The bark of neem plant [Azardirachata indica Linn (Meliaceae)] has been widely used as a traditional medicine for many centuries in tropical countries. Earlier studies have confirmed that neem bark extract contains some substance with strong anti-inflammatory, anti-viral, anti-oxidant and many more properties. NBE is also significantly used in skin diseases. According to Ghimeray et al. leaf and bark extracts/fractions of neem grown in the foothills have significant antioxidant properties [38].

An In-vitro antiviral activity of neem (*Azardirachta indica L.*) bark extract against herpes simplex virus type-1 infection, study was done in August 2010 [21]. Herpes simplex virus (HSV) infections are extremely widespread in the human population. The virus causes a broad range of diseases ranging from labial herpes, ocular keratitis, genital disease and encephalitis. The herpes simplex virus is categorized into 2 types: herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). HSV-1 is mainly transmitted by oral-to-oral contact to cause oral herpes (which can include symptoms known as "cold sores"). HSV-1 can also be transmitted from oral or skin surfaces that appear normal and when there are no symptoms present but can also cause genital herpes. An estimated 3.7 billion people under age 50 (67%) have HSV-1 infection globally. Most oral and genital herpes infections are asymptomatic. Herpes infections are most contagious when symptoms are present but can still be transmitted to others in the absence of symptoms [39]. The result obtained from the study done at 2010 that an aqueous extract preparation from the barks of neem plant [*Azardirachta indica]* acts as a potent entry inhibitor against HSV-1 infection into natural target cells. The neem bark extract (NBE) significantly blocked HSV-1 entry into cells at concentrations ranging from 50 to 100 µg/ml. The blocking activity of NBE was observed when the extract was pre-incubated with the virus but not with the target cells suggesting a direct anti-HSV-1 property of the neem bark. Further, virions treated with NBE failed to bind the cells which implicate a role of NBE as an attachment step blocker. Cells treated with NBE also inhibited HSV-1 glycoprotein mediated cell to cell fusion and polykaryocytes formation suggesting an additional role of NBE at the viral fusion step [20,21]. These finding open a potential new avenue for the development of NBE as a novel approach for safe mass sanitization formulation.

As a cross reference of this approach of using neem bark extract which has been proved to be effective against HSV-1 infection, has to be mentioned. The researchers in Knipe Lab of department of microbiology, Blavatnik Institute, Harvard Medical School have decided and constructed to use the HSV-1 virus vector to develop covid vaccine. This vector, named HSV-1 d106S can express the bacterial, viral, cancer gene products to develop immunity in

humanized mice. Among several important reason of using the HSV-1 vector, one of the important reason behind it is HSV replication-defective vectors induce a strong Th1 helper T cell response. As such, this vector induces a strong priming immune response. So, HSV-1 d106S can avidly be used to rapidly develop COVID-19 vaccine to fight against global pandemic. Thus the NBE can be an effective & safe Mass-sanitizing solution. [40]

CONCLUSION:

In the present scenario of COVID-19 outbreak, India is in unlock phase and there is no treatment or vaccination till date to overcome this infection disease process, preventive measures are the only concern. In this circumstance large-scale disinfection and safe mass sanitization can be multi-barrier approach for routine practice. Though, chemical mass sanitization is not safe as stated by Heath Ministry of India. So, there is a need of using herbal formulation is to spray over human body to disinfect them with no adverse effects. Safety, Toxicities, and LD50 Values of the NBE herbal formulation has been done and tested in the laboratory. It is a safe and effective sanitization formulation with lesser or no side effects. These finding open a potential new avenue for the development of NBE as a novel approach for safe mass sanitization formulation. This would be an effective biosafety system which will act as a multi-barrier approach to break the chain of the virus and maintain virus free campus. Hence this system promotes a healthy hygiene and protection for future.

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