

FORMULATION OF HERBAL SANITIZER FROM CAESALPINIA BONDUCELLA

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ABSTRACT

Medicinal plants are the main source of pharmaceuticals and healthcare products. In the present study, our goal was to investigate the phytoconstituents in the leaf extracts of *Caesalpinia bonducella* and their antimicrobial activity. The phytochemicals analysis of leaves powder of *Caesalpinia bonducella* was carried out by using extraction method. *Caesalpinia bonducella* leaves contain Terpenoids & Phenolics 4.490%, Alkaloids 0.885%, Q. Alkaloid & N-oxides 25.745 %, fats and waxes 8.850% and Fibers 60.050%. Antimicrobial studies showed that the above extracts had considerable activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, *Aspergillus niger* and *Penicillium chrysogenum*. The percentage of fats and waxes, terpenoids and phenolics, Q. Alkaloid & N-Oxides was observed good. Its beneficial effect on animals as well as human health may be derived from its antibacterial and antifungal

properties to afford protection against various diseases. It might be an alternate to synthetic antibiotics available in the market.^[1] Hand hygiene is of unconditional importance as it may be contaminated easily from direct contact with airborne micro-organism droplets from coughs and sneezes. To date, most of the effective hand sanitizer products are alcohol-based formulations containing 62%-95% of alcohol as it can denature the proteins of microbes and the ability to inactivate viruses. *Caesalpinia bonducella* leaves has significant antidiarrhoeal properties. The methanol extract and other three fractions of the *C. bonducella* leaves possess potent antibacterial activities along with moderate cytotoxicities that may lead to new drug

development.^[2]

KEYWORDS: *Caesalpinia bonducella*, quaternary alkaloids, terpenoids and phenolic, phytochemical, anti-microbial activity.

INTRODUCTION

From prehistoric times, it has been an aim to use herbal plants as drugs for human beings but only 5% of the total 3, 00,000 plant species has been studied scientifically for their medicinal purposes. More than 30% of all prescription drugs are derived from plant origin in the industrialized countries. Ayurveda, Unani, Homeopathy and Siddha are the traditional systems of medicines which mainly depend on the natural products. People below poverty line in the undeveloped and developing countries of Asia and Africa are suffering from health problems connected with the consumption of mycotoxin contaminated grains and cereals.^[1]

Given the dangers imposed by this disease, the Centre for Disease Control and Prevention (CDC), the United States has promoted and encouraged hand hygiene through handwashing or use of hand sanitizer. Hand disinfectants are commercially available in various types and forms such as anti- microbial soaps, water-based or alcohol- based hand sanitizers, most often used in hospital settings. Different types of delivery systems are also formulated for instance, rubs, foams, or wipes. The World Health Organisation (WHO) recommends alcohol based hand sanitizer (ABHS) in line with the proven advantages of their rapid action and a broad spectrum of microbicidal activity offering protection against bacteria and viruses but alcohol based sanitizer or any other disinfectant damage hands and other body parts by using alcohol based sanitizer or any other disinfectant. Herbal medicine has been used for thousands of years. It is estimated that 80% of world population depend on traditional herbal medicine for primary health care. The phytoconstituents present in herbal medicine have better compatibility with the human system.^[2] *Caesalpinia bonduc* (C. Bonduc) classified under the family of caesalpiniaceae. Commonly it is called as fever nut. Whole plant of C. Bonduc use as herbal medicine. The plant is found in all over India. C. Bonduc is largely used for its antiperiodic, antipyretic, antimalarial properties. It is also described to have antioxidant, antibacterial, antitumor and antidiabetic activities. Leaves of the C. Bonduc show the antimicrobial activity. Leaves powder was carried out by using extraction method and leaves extracts were screened for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and others. The aim of the work is to prepare and evaluate non-toxic, non-habitual Hand Sanitizer from *Caesalpinia bonduc*.^[3]

Drug Profile



Synonym -: Fever Nut, Sagargota, Gray Nicker, Latakaranj

Biological Source-: It is dried entire plant of *Caesalpinia Bonduc* Linn.

Family-: Leguminosae or Caesalpiniaceae

Chemical Constituents-: Chemical constituents such furanditerpenes, phytosterinin, B-sitosterol, flavonoids, bonducellin,

Pytochemicals-: Phytochemical compounds aspartic acid, arginine, citrulline and B-carotene. as alkaloids, saponins, tannins, flavonoids and steroids have been known to be biologically active and thus partially responsible for the antimicrobial activities of plants, hence their use in traditional medicine.

Caesalpinia bonducella is an important medicinal plant uses against different types of diseases. The study investigated the antimicrobial activities of the methanol extract of *C. bonducella* leaves.^[4]

Preparation of Sanitizer

Materials: *Caesalpinia Bonduc*, Basil water, Glycerin, Fragrance, Camphor were the materials utilized for formulating hand sanitizer as a non-alcohol based Sanitizer.^[5]

Method

1. Extraction of *Caesalpinia Bonduc* leaves.
2. Collection & Authentication of *C. Bonduc* leaves.
3. Drying of leaves in shed drying to make course fine. Extraction-Maceration (To get methanolic extract 90% methanol in 4:1 ratio for 24 hrs.)
4. Filtration (Filtrate was taken) Extract & Marc.
5. Evaporation of methanolic extract to get *C. Bonduc* leaves.

6. To study the anti bacterial activity of C. Bonduc leaves extract.
7. Preparation of herbal sanitizer (By using herbal extract and suitable excipients).
8. To evaluate anti microbial activity of C.Bonduc leaves as compared to C. bonduc extract and other marketed sanitizer.
9. Packing and Labelling. [Also we use agar culture media to check the anti-microbial activity of C. Bonduc herbal Sanitizer]



Agar Medium

Agar medium is a gelatinous substance derived from seaweed, used in laboratories for culturing micro-organisms like bacteria, fungi, and other small organisms. It provides a stable surface for microbial growth and allows researchers to isolate, grow, and study different species. Agar medium's solid form helps maintain the integrity of colonies for further analysis and experimentation.^[6,7]

Preparation of agar medium

Preparation of agar medium involves mixing agar powder with nutrients such as peptones, salts and sugars in water. The mixture is heated to dissolve the agar sterilized and poured into Petri dishes or tubes. Once solidified, the medium becomes a gel that provides a surface for the growth of microorganisms. Various variations exist based on specific requirements, including selective or differential media tailored to support the growth of certain organisms or distinguish them based on characteristics such as color changes or responses to specific chemicals.^[6,7]



Antimicrobial Activity^[1,8]

To carry out antimicrobial activity, agar diffusion method was used and the diameter of growth inhibition zone surrounding the antibiotic disc was measured to determine it. At first the sterilized Muller- Hinton agar medium 20 ml was poured into a sterile petriplate. Then the plate was covered and allowed to gel. The sterile cotton swab was dipped into the culture suspension of bacteria. The agar surface of each plate was inoculated by using the swab and ensuring the even distribution of the organism over the agar surface. The agar surface was allowed to dry for ten minutes. A sterile filter paper disc was picked up by the outer edge with sterile forcep and dipped the opposite edge of the disc in the prepared solution of the extract with concentration 100 µg/ml. The disc was placed near the edge of the agar surface of the inoculated plate. The incubation of the plates was carried out at 37°C for 24 hours. The transparent meter rule was used to measure the zones of inhibition. Chloramphenicol with concentration 10 µg/ml was used as standard.

Results and Discussion on Types of Hand Sanitizer

Hand sanitizer can generally be categorized into two groups: **alcohol-based** or **alcohol-free**. An ABHS may contain one or more types of alcohol, with or without other excipients and humectants, to be applied on the hands to destroy microbes and temporarily suppress their growth.^[5] ABHS can effectively and quickly reduce microbes covering a broad germicidal spectrum without the need for water or drying with towels. Nevertheless, there are a few shortcomings with the effectiveness of ABHS, such as its short-lived antimicrobial effect and

weak activity against protozoa, some non-enveloped (non-lipophilic) viruses and bacterial spores.^[5] But ABHS damage our hand sensitivity and give some allergy effect.

On the other hand, the alcohol-free sanitizer makes use of chemicals with antiseptic properties to exert the antimicrobial effects. These chemicals have a different mode of action and function according to their chemi functional groups. As \uparrow they are non-flammable and often used at low concentrations, they are relatively safe to use among children as compared to ABHS.

Non-alcohol based hand sanitizer is available in different dosage forms, namely gel, liquid and foam. As each type has its own characteristics, a study was conducted to understand the impact on sensory attributes that may affect user's acceptance of the product and ultimately influence usage leading to hand hygiene compliance. The overall result showed that gels and foams are more widely accepted compared to liquid, especially in terms of handleability, though the latter left a high clean feeling and took a shorter time to dry.

However, there are several limitations associated with the sprays, including overspray, breathed by patients and flammability. Ready-to-use alcohol "Hand Sanitizing Wipes (HSW)" is a pre-wetted towelette containing disinfectants, antiseptics, surfactants, etc. in a sealed package ready for use in topical disinfection. Also soap, handwash etc.^[11]

Alcohol and Soaps

Keeping hands clean is a fundamental and essential step to avoid getting sick while limiting the transmission of germs to others. CDC (Centre of disease control and prevention) recommends handwashing with soap and water whenever possible as it remarkably reduces the amount of all types of microbes and dirt on the skin surface. Both the soaps and non alcohol-based sanitizers work by dissolving the lipid membranes of microbes, thereby inactivating them. Thus, the sanitizer serves as an alternative when the soap and water are not readily available.^[14]

Physiology of Hand Skin

The skin is composed of three main layers: a superficial epidermis (50–100 μm), a middle dermis (≈ 2 mm), and an innermost hypodermis (1–2 mm). It constitutes the first line of defence against invading microorganisms while providing protection against mechanical impacts and preventing excessive loss of water from the body.

The vital barrier function of the skin resides primarily in the uppermost epidermal layer, the stratum corneum (SC). The SC contains layers of corneocytes that are terminally differentiated from keratinocytes that make up the basal layer of epidermis.

The adjacent corneocytes are interconnected by membrane junctions called corneodesmosomes to enhance the cohesion of the SC.^[15] The lipids that are derived from the exocytosis of lamellar bodies during terminal differentiation of keratinocytes will fill up the intercellular spaces between the corneocytes, and they play a role in maintaining the cutaneous barrier function. The layer underneath the SC is known as the keratinized stratified epidermis. It consists of melanocytes that produce melanin, a skin pigment that provides skin with its color and protects the skin from ultraviolet radiation. Apart from that, Langerhan's cells, which are involved in the immune response and Merkel cells that are responsible for light touch sensation, can also be found within this layer.

Though the skin serves as a barrier that protects one against harmful microorganisms, it hosts a wide array of beneficial bacteria such as *Staphylococcus epidermis*, *Staphylococcus aureus*, *Micrococcus* spp., *Propionibacterium* spp. and *Corynebacterium* spp. These bacteria may help to prevent the colonization of pathogenic microbes by either competing with them for nutrients or stimulating the skin's defence system. Under normal circumstances, they exhibit low pathogenicity. However, when the skin flora distribution is disrupted, for example, due to the long-term use of topical antibiotics or frequent hand washing, they may become virulent. To reduce the incidence of infection, the microbiota balance is restored and maintained through constant skin regeneration. The whole process takes about 28 days, starting from the mitotic division of basal epithelium to desquamation. When the dead keratinocytes in the SC are sloughed off, it takes away the microbes that colonized the skin surface. This continuous process significantly limits the invasion of bacteria while achieving a balanced growth among the microbial populations.^[15]

The Adverse Effects of Alcohol-Based Sanitizer or Handwashing Soaps

The most commonly reported skin reactions with the use of ABHS are irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). The symptoms of ICD can range from mild to debilitating with manifestations like dryness, pruritus, erythema and bleeding, if severe. As for ACD, the symptoms can either be mild and localized or severe and generalized, with most severe forms of ACD being manifested as respiratory distress or other anaphylactic symptoms. Sometimes, it may be difficult to distinguish between ICD and ACD due to the

overlap and similarities of symptoms.

Hand hygiene products such as sanitizer and soaps can be damaging to the skin through several mechanisms: denaturation of the stratum corneum proteins, alteration of intercellular lipids, decrease in corneocyte cohesion and reduction of stratum corneum water-binding capacity. The biggest concern is the depletion of the lipid barrier, especially with repeated exposure to lipid-emulsifying detergents and lipid-dissolving alcohols as it may penetrate deeper into the skin layers and change the skin flora, resulting in more frequent colonization by bacteria. In order of decreasing frequency of ICD including handwashing soaps are iodophors, chlorhexidine, chloroxylenol, triclosan and alcohol-based products. Among the alcohol-based formulations, ethanol has the least skin-irritant property compared to n-propanol and isopropanol.^[11] There are, however, other contributing factors that increase the risk of ICDs such as lack of use of supplementary emollients, friction due to wearing and removal of gloves and low relative humidity. ABHS also has a drying effect on hands which can further cause the skin to crack or peel.

On the other hand, ACD is caused by allergic reactions towards certain agents in the formulations such as iodophors, chlorhexidine, triclosan, chloroxylenol and alcohols. Individuals with allergic reactions to alcohol based preparations may have true allergy to alcohol or allergy to impurity, aldehyde metabolite or other excipients like fragrances, benzyl alcohol, parabens or benzalkonium chloride.^[11]

PH Evaluation

The pH values of the formulated hand sanitizer gels were measured using a digital pH meter. The study was conducted to check the neutralization of different prepared formulations. The ideal standards for a pH value of a topical dosage form should be within the broad pH range of the skin, i.e., 4.0 to 7.0, in order to avoid skin inflammation and irritation.^[11] The pH measurements all prepared formulations were slightly acidic, with pH values around 3.9. This might be due to the large proportion of aloe vera (90% v/v) with a natural acidic pH (4.0–4.5).

Acceptability Test (Skin Irritation Study)

The skin irritation study was performed. According to the pH evaluation results, viscosity, spreadability, and antimicrobial activity. Ideal hand sanitizers should possess a pleasant smell, feel comfortable upon use, be easy to apply and not sticky, and have an excellent

antimicrobial activity.

The skin irritation study results showed that the hand sanitizer was very well-tolerated, and did not produce any sign of irritation or skin redness after being applied on the skin. However, a minimal sense of itching was reported in few volunteers whom already suffered from a skin condition, namely eczema, and demonstrated redness. Hand sanitizer gel was applied again and no side effects were reported.^[12]

Glycerine

Glycerin is a natural humectant, meaning it pulls moisture from the air to your skin to prevent it from drying out. This makes it a vital ingredient for soaps and sanitizers. When you clean your hands with soap or alcohol, you are stripping your skin of its natural oils. This can lead to drying and irritation.^[5]

Glycerin is a natural by product of the soap making process. When using natural soap, glycerin helps your skin to maintain moisture while the soap washes away dirt, germs, and harmful bacteria.

Glycerin is also a key ingredient in our hand sanitizer. Alcohol is incredibly effective at inactivating viruses and killing harmful bacteria on the skin. But alcohol evaporates quickly which means that it can also be extremely drying. That's the reason you should never clean your hands with alcohol alone without also moisturizing them. We use glycerin in our hand sanitizer so that you can effectively clean your hands without the drying and cracking. Glycerin pulls moisture from the air to combat the drying effects of the alcohol. This means that your skin will be left moisturized after using our sanitizer.^[13]

Here are the top skin benefits of Glycerin

Moisturizes your skin: You likely have several products with glycerin in them. Glycerin is often found in moisturizers as it helps to maintain moisture on your top layer of skin. Glycerin is also found in cleansers for the same reason. We use glycerin in our soaps and sanitizers to act as a moisturizer.

Helps protect your skin against irritation: Alcohol and other cleansers can irritate your skin and exacerbate issues such as eczema, and psoriasis. Glycerin helps protect your skin against these irritants and maintain moisture.

Protects your skin's moisture barrier: The top layer of skin, also known as the epidermis, protects the skin from damage and irritation. Glycerin can help you to maintain your skin's moisture barrier and prevent you from having dry, flaky and damaged skin.^[5,13]

RESULTS

Phytochemical analysis and antimicrobial activity of *Caesalpinia bonducella* were carried out in this research. The preliminary phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoides, carbohydrates, proteins, phenolic compounds and quinine (Table 2). Saponins were not detected in the ethanol extract. These constituents present in the leaves extracts have good therapeutic value.

Table 1: Bacterial culture.^[1]

S.No.	Name	Type
1	Bacillus subtilis	Gram positive
2	Staphylococcus aureus	Gram positive
3	Escherichia coli	Gram negative
4	Klebsiella aerogenes	Gram negative

Table 2: Preliminary Phytochemical Analysis of extracts of *Caesalpinia bonducella*.^[1]

S. No.	Phytochemical constituents	Water extract	Ethanol extract
1	Saponins	++	-
2	Tannins	+	+
3	Alkaloids	+	+
4	Flavonoids	+	++
5	Carbohydrates	++	++
6	proteins	++	++
	Phenolic compounds		
7		+	+
8	quinine	+	++

Where,

++ = High concentration

+ = Low concentration

- = Absent

Secondary metabolites of the plants like alkaloids, terpenoids and glycosides play the role of protective agents against different pathogens like insects, fungi or bacteria. They also function as growth regulatory molecules such as hormone like substances. Consequently, they are used as potential anticancer drugs by direct cytotoxic activity against cancer cells or by reducing the tumor development process.^[23] The phytochemical analysis study showed the

presence of 4.49% terpenoids & phenolics.

In Pharmaceutical and food industries, terpenes are used as medicines and flavor enhancer because of their potentials and effectiveness. As antibiotic resistant bacteria are being increased globally, terpenes are important.^[1] The group of terpenoids exhibits various pharmacological activities like anti-viral, anti-malarial, anti-inflammatory and anti-cancer activities. It also inhibits cholesterol synthesis.^[1] (Table 3)

Table 3: Phytochemical Analysis of *Caesalpinia bonducella*.^[1]

S.No.	Parameters	% Composition
1	Fats and waxes	8.85 %
2	Terpenoids and phenolics	4.49%
3	Q. Alkaloid & N – Oxides	25.745 %
4	Alkaloids	0.885%
5	Fibers	60.05 %

The result of the research showed that the Hand Sanitizer of *C. Bonduc* gives good anti microbial activity which protect against gram positive and gram negative bacteria. *C. bonduc* hand sanitizer was include in the gel to prepare a superior antimicrobial product with slight or no adverse effects and higher acceptability for human skin.

DISCUSSION

Caesalpinia bonduc (LATAKARANJ) can become the best source of medicine in future. However, more research should be directed in future chances to evaluate the effectiveness against more bacterial species, yeast, and fungus.

CONCLUSION

Although a diverse number of modern medicines are used for curing acute and several illnesses, herbal medicines have maintained their importance. Our survey of literature concluded that *Caesalpinia Bonduc* is used traditionally since ancient times for many medicinal purposes. During the present work methanolic and ethyl acetate extracts of *C. Bonduc* leaves revealed antimicrobial properties. Methanolic extract showed better antibacterial activity in vitro, and can be developed as antibacterial agent for curing infectious diseases. However, further works are required to explore other pharmacological activities of *C. Bonduc* leaves both in vitro and in vivo.

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