

## Formulation and evaluation of Herbal Sanitizer by using Curcumine, Neem and Tulsi leaves.

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### Abstract

**Objective:** the main intention of this research is to prepare curcumin and Neem based herbal gel sanitizer by minimizing the alcoholic usage and to evaluate the anti-bacterial activity of this herbal sanitizer. **Methods:** curcumin and Neem gel sanitizer was prepared from the ethanolic extract of *Curcuma longa* and *Azadirachta indica* ( along with addition of gel base prepared from HPMC-E15. **Results:** The curcumin and Nee gel sanitizer was formulated and evaluated for its phytochemical constituents present in curcumin and neem, organoleptic properties, irritancy test and the efficiency of anti-bacterial activity of curcumin was also evaluated and it is safe and effective against pathogens. **Conclusion:** As a natural herb, curcumin and neem which was a household ingredient could also be effectively formulated as a sanitizer that reduces the side effects of alcoholic sanitizer products and is a best source that acts effectively against a numerous pathogens.

Keywords: curcumin, HPMC-E15 (hydroxypropyl methylcellulose), Pathogens,

### INTRODUCTION

Corona virus is a single-stranded RNA virus with a diameter of about 80–120 nm. It belongs to different genus like  $\alpha$ -corona virus,  $\beta$ -corona virus,  $\delta$ -corona virus and  $\gamma$  – corona virus. SARS-CoV-2 comes under the genus  $\beta$ -corona virus. 4 The emergence of the pandemic COVID-19 has lead to health concern in the public and rapid usage of hand sanitizers is being observed. As it can be effective tool of controlling the infection. Recent studies revealed that the transmission of COVID is possible through the mist and the virus can sustain and may become contagious on surfaces about 9 days Hence, it is very important to breakout this conveyance of virus through isolation and by the usage of infection control tools 3. Effective usage of hand sanitizers and face masks could minimize the spread of the virus. According to the analysis of CDC about 2 million people are getting affected by certain infections acquired through the hospitals per year (12). Due to danger imposed by this disease, the CDC of the United States has encouraged and promoted the hand sanitizers usage for the sake of maintenance of hand hygiene. The availability of Hand disinfectants are in such forms like Non-alcohol based, alcohol-based, herbal sanitizers(15).

Hand sanitation is a crucial step in controlling the infectious virus in the healthcare systems.(9) Numerous hand-sanitation products were accessible outside as they became an emergent rise for the maintenance of hand-hygiene can be seen(14). Besides regular soaps, hand sanitizers which consists of different chemical agents with effective anti-microbial properties(such as chlorhexidine, iodophors, quaternary ammonium compounds, triclosan) without the use of water or hand drying materials are used rapidly these days.3

Alcoholic formulations are very effective in various situations. They exterminate various types of pathogens(8) but they are not much efficacious in eradicating the organisms such as *Clostridium difficile* or *Bacillus anthracis*. In addition rapid usage of these alcohol-based sanitizers can cause flammability, endocrinal disruptions etc.(1) Moreover the culture of intentional ingestion of alcohol-containing sanitizers has become popular during this pandemic(1)

This brings out the case of non-alcohol based sanitizers or herbal based sanitizers which is becoming an option. Medicinal plants consist of abundant secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, as these are found to possess greater anti-microbial activity(16). Taking this into consideration, the literature screening was performed for different herbs with the anti-microbial properties and founded them (such as *Curcuma longa* (turmeric), *Mentha piperita* (peppermint leaves), *Eugenia caryophyllus* (clove flower buds), and *Azadirachta indica* (neem leaves)7. Before the discovery of advanced medicine, herbs are the crucial remedy used for various disease treatments. This arrivals of different antibiotics; microbes begin to develop resistance to these antibiotics. This brings the researchers towards the herbs those are abundant in microbial activity(15)

This research is carried out by formulating an herbal sanitizer with the extract of *Curcuma longa*.and *Neem* The ethanolic extract of turmeric and Neem has strong anti-microbial properties against the growth of certain microbes such as *Pseudomonas aeruginosa*, *Bacillus cereus* and *Proteus mirabilis*. It also has good anti-fungal activity against *Trichophyton longifusus*. External application of curcumin and neem was found to decrease variegation and onset of skin tumors.(7)

## MATERIALS AND METHODS

### Materials

Turmeric was obtained from local market.Neem and Tulsi Leaves Obtained from Garden of Maharashtra Institute of pharmacy,betada,HPMC-E15 ,Glycerine and perfuming agents was obtained from Samar chemicals.

### Method

#### Plant material processing:

Newly harvested rhizomes of curcumin and leaves of neem and Tulsi are collected, cleaned and dried under sunlight. After the process of drying were collected and size reduced by passing through the mesh of suitable size.(2)

**EXTRACTION PROCESS:**

Powdered rhizomes of curcumin and Nemm leves were extracted with 95% ethanol through the process of maceration for about 24 hours. The obtained crude extract was filtered, concentrated and collected.

**PRELIMINARYPHYTOCHEMICAL ASSESSMENT OF CRUDE EXTRACT:**

The prepared curcuminand neem extract was assessed for the presence of different phytoconstitents such as carbohydrates, proteins, alkaloids, flavonoids, tannins and saponins using standard preliminary tests.

**CARBOHYDRATES:** The curcumin and Neemextract is dissolved in 10ml of purified water, filtered and was subjected to the tests for carbohydrates.(17)

**Molisch test:** 2ml of curcumin and neem extract and was added into the test tube and few drops of molisch reagent was added to extract. To this 2ml of conc.H<sub>2</sub>SO<sub>4</sub> was dropped out from the walls of the test tube. Formation of a violet color ring at the junction of two liquids was observed.(17)

**PROTEINS:** The processed curcuminand neem extract was dissolved in 10ml of water, filtered and test for proteins was carried out. (17)

**Biuret test:** 2ml of filtered solution is treated using a drop of 2% copper sulphate solution. Later on addition of few drops of ethanol (95%). Followed by the addition excess of potassium hydroxide pellets. Pink color formation in ethanolic layer indicates the protein presence. 17

**ALKALOID:** the solvent free extract of required17 quantity was added to 3ml of dilute HCL and then filtered. The filtrate was then carried out for the test of alkaloids. 17

**Mayer's test:** To 1ml of filtered extract 2 drops of Mayer's reagent is added from the walls of the test tube. The presence of white or creamy precipitate includes for the alkaloid presence. 17

**Wagner's test:** To 1ml of filtrate 2ml of Wagner's reagent was added from the walls of the test tube.Presence of reddish-brown precipitate confirm positive for the above test.(17)

**Dragendroff's test:** To the 1ml of filtered extract 2ml of Dragendroff's reagent is added. Formation of yellow color precipitate confirms were positive test17.

**GLYCOSIDES**

**Borntrager's test:** Prepared curcumin and Neem extract was boiled with dilute H<sub>2</sub>SO<sub>4</sub>, filtered and the addition of chloroform was done and shaken well. The separation of organic layer was observed to which ammonia is added slowly. The appearance of pink or red color in ammonical layer indicates positive for the test.(13)

**TERPENOIDS**

**Salkowski test:** To the extract add 2ml of chloroform and 3ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added carefully. A reddish brown color formation indicates presence of terpenoids.13

**FLAVONOIDS:** To the extract 1.5ml of 50% methanol was slowly added. The above solution is warmed and magnesium metal was added onto it. To this, addition of concentrated sulphuric acid and red color was observed indicating for the presence of flavonoids.13

**TANNINS:** To 0.5ml of extract ,water and few drops of ferric chloride was added. Presence of blue color indicates for tannins presence.(13)

**SAPONINS:** The processed curcuminand neem extract was shaken with 5ml of water and then heated for boil. Frothing shows up for saponins presence. (17)

**FORMULATIONS**

**Table no 1:Formulation of Herbal Hand Sanitizer.**

Sr No	Ingredients	Quantity (ml)		
		F1	F2	F3
1	Neem extract	1	2	3
2	Curcuma Extract	2	1	3
3	Tulsi extract	1	2	3
4	HPMC-E15	0.1	0.1	0.1
5	Glycerine	5	5	5
6	Perfuming agent	q.s	q.s	q.s
7	water	Upto 20	Upto 20	Upto 20

A gel base was prepared using HPMC-E15 and water and transferred into the motor. Add extract of Curcumine, Neem and Tulsi into gel base, filter the mixture, To the filtrate add other ingredients like glycerine and rose water and make up volume with water.

**RESULTS AND DISCUSSION:**

**RESULTS:**

**PHYTOCHEMICAL SCREENING**

The phytochemical evaluation of ethanolic extract of curcumin and Neem results for the existence of secondary metabolites such as carbohydrates, proteins, alkaloids, terpenoids, tannins and flavonoids but the absence of saponins and glycosides. These secondary metabolites serve as competitive weapons against the bacteria, fungi, amoebae and insects.

Table no 2. Phytochemical screening of herbal hand sanitizer

Sr.No.	Test	Observation
1	Carbohydrate	+ve
2	Protein and amino acid	+ve

3	alkaloids	+ve
4	Glycosides	-ve
5	Terpenoids	+ve
6	Tannis	+ve
7	Saponines	-ve
8	Flavonoids	+ve

**Evaluation Parameters:**

**Organoleptic properties:** The color, odour and clarity of the formulation F1,F2,F3 were carried out.

**pH:** pH of sanitizer was carried out through pH meter and it was observed that the pH of sanitizer was optimum that is exactly 6.9.

**Skin Irritant test:** Few healthy Volunteers were selected. The formulated curcumin-Neem sanitizer was applied on their palm and the time was noted down. Irritancy and itching were checked(11)

**Evaporation time:** Few healthy Volunteers were selected. The curcumin sanitizer was applied on their palm and the time of evaporation was noted down. All the evaluated parameters were provided in the below table-03

Table no.03 Evaluation Parameter of Herbal hand Sanitizer.

Sr.No.	Parameter	Observation		
		F1	F2	F3
1	Colour	Light green	Light green	Dark green
2	Odour	Characteristics	Characteristics	Characteristics
3	PH	6.8	6.8	6.9
4	Irritancy test	No irritation	No irritation	No irritation
5	Evaporation Rate	20sec	18sec	15sec

**Evaluation of anti-bacterial activity:** The evaluation of anti-bacterial activity of curcumin gel sanitizer was carried out using different solvents against the strain of aerobic and anaerobic micro-organisms by performing cup-plate method. The nutrient agar medium used for the culture media was provided in the below table-04.

Table No 04: Composition of Nutrient Agar medium.

Sr.No	Ingredients	Quantity(g)
1	Beef extract	0.2
2	Yeast Extract	0.4
3	peptones	1
4	Sodium Chloride	1
5	Agar	1.5
6	Distilled Water	q.s for 100ml

To perform the test, the Petri-plates required for the test must be pre-sterilized to avoid cross-contamination. Petri-plates were pre-sterilized at 160°C in hot air oven. Then, Prepared nutrient agar medium was autoclaved at 121°C for about 30 minutes. Under aseptic conditions culture media was transferred into nutrient agar medium and this entire media is transferred into petri-plate. After, uniform spreading the petriplates were kept aside until the medium gets solidified. Then, the plates were taken out and cups or wells were made by using cork borer. Then, the standard 95% ethanolic extract of turmeric and the test sample was added into the cup carefully in aseptic conditions. Then both the plates are incubated for 24hrs at 37°C. After, the incubation period, the zone of inhibition was measured on the Petri plate.

The anti-bacterial evaluation of the formulated curcumin gel sanitizer was found to be safe and effective against the bacterial species such as *E.coli* and *Bacillus subtilis*. As it can be seen that anti-microbial effect of curcumin sanitizer is more efficacious in inhibiting the growth of the microbes as that of the standard i.e ethanolic extract of turmeric. The results were provided in the below table-05.

Table No-05 Antibacterial Evaluation of Herbal hand sanitizers

Sr.No	Anti bacterial test	Zone of inhibition(standard)	Zone of inhibition (formulation)mm		
			F1	F2	F3
1	<i>E.coli</i>	18	15	14	13
2	<i>B. Subtilis</i>	14	10	11	09

**DISCUSSION**

The curcumin-Neem gel hand sanitizer was evaluated for its phytochemical screening of active constituents, and also the determination of active constituent of curcumin and Neem and evaluation of organoleptic properties of the formulated herbal gel sanitizer and checked for the irritancy test and the evaporation time. It was found that the above formulation consist of the green colour with the gel consistency and smooth texture. It was also tested for its anti-microbial activity against *E.coli* and *B.subtilis* and has shown a significant effect against these microbes. The above formulation consists of the ethanolic herbal extract to minimize the alcohol usage in the formulation of the herbal sanitizer.

**CONCLUSION**

Hands are the common platform for the pathogenic transmission to the people. The proper maintenance of hand hygiene can protect the health from various contagious pathogens especially for the prevention of COVID-19 transmission. WHO recommendation for the usage of alcohol based-sanitizers during this pandemic for the prevention of the disease transmission. But the rapid usage of these alcohol-based sanitizers causing the endocrinal disruptions, flammability and alcohol poisoning if ingested and the intentional ingestion of alcohol containing sanitizers all these were major cases to be considered.

It may be concluded that Formulation no 3 (F3) of curcumin and Neem gel sanitizer has a significant role as it shows significant effect against specified organisms. Thus, there is immense care in establishing the usage of turmeric and Nem gel sanitizer as a measure to control the multi-drug resistant microbes. This herbal gel sanitizer is also based as an alternative for the

chemically prepared sanitizers containing active silver nitrates. Natural herbal sanitizers are very efficacious, environmental friendly, and inexpensive.

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