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Formulation of an Herbal Substitute for Chemical Sanitizer and its Evaluation for Antimicrobial Efficiency

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Abstract : The research was carried out to study the antimicrobial properties of aqueous extracts of perennial plants leaves, that are Eucalyptus, Neem (*Azadirachta indica*) and Sadabhar *(Catharanthus roseus)* using a plethora of techniques such as Finger Imprint technique, Agar well diffusion method and Effect on micro flora. By amalgamating all the results the efficacy of the aqueous extracts observed was in the order, Eucalyptus >Sadabhar*(Catharanthus roseus)*> Neem(*Azadirachta indica*), subsequently. Based upon these results hand sanitizer was prepared using eucalyptus, rose extract and glycerin. The hand sanitizer developed was screened for its antimicrobial activity. The activation time period for herbal sanitizer was observed to be 5minutes while that of alcohol based sanitizer was 1 minute theoretically. To conclude, it can be stated that Eucalyptus leaves have natural antimicrobial properties that can be exploited to eradicate the microbial problems.
Keywords : Aqueous extract, Eucalyptus, Neem(*Azadirachta indica*), Sadabahar*(Catharanthus roseus)*, Antimicrobial activity, Hand sanitizer.

Introduction

Sanitizers are substances that acts as both cleaning and disinfecting agents. Hand sanitizer is a liquid generally used to sanitize animate articles [1], [2]. Alcohol-based sanitizers generally contain some combination of iso propanol, ethanol or n- propanol [3]. Most effective versions contain 60 to 95% alcohol [3]. Sanitizers work by destroying the cell wall of microbes or interfering with the metabolism. As alcohol based hand sanitizers showed following ill effects:

- Alcohol based sanitizers may kill both bad and beneficial bacteria.
- May act as endocrine disruptors and may be a factor in early puberty
- Can result in alcohol poisoning if ingested. Therefore should be kept out of reach from children or used under adult supervision only.

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- Being flammable produce hazardous waste, and they must be disposed of and stored according to Occupational Health and Safety Administration (OSHA) guidelines.
- Due to too frequent use, skin may lose natural oils may lead to drying and cracking. Dehydrated skin can be unattractive and irritating, dry, cracked cuticles and skin can offer an entry point for germs to enter the body and cause infection.

Natural sources like herbs, trees, essences and extracts are known to contain numerous bioactive products that shows antimicrobial, analgestic, anti-oxidative and anti-inflammatory effects. [4], [5]. The aim of this research was to explore the antimicrobial activities of aqueous extracts of Eucalyptus, Neem and Sadabaharin order to substitute the chemical products with the eco- friendly ones.

Eucalyptus a native plant of Australia, belongs to a diverse genus of flowering trees and shrubs in the myrtle family, called Myrtaceae. [4],[6],[7]. Eucalyptus species are cultivated widely in the tropical and temperate world, including the Americas, Europe, Africa, the Mediterranean Basin, the Middle East, China and the Indian subcontinent. [4],[6], [7]. This plant is a rich source of poly phenols and Terpenoids and the base composition (70 to 80 mg/ml) of the leaves are the Eucalyptol or Cineole[4], [8]. Some eucalyptus species have attracted attention from microbiologists, horticulturists, global development researchers, and environmentalists because of desirable traits such as being fast-growing sources of wood, producing oil that can be used for cleaning and as a natural insecticide and antimicrobial activity.

Neem *(Azadirachta indica*) also known as Indian lilac, belongs to the mahogany family Meliaceae. It is native to the Indian landmass, i.e. India, Nepal,

Pakistan, Bangladesh, Sri Lanka, and Maldives, typically grown in tropical and semi-tropical regions[9]. Leaves contain compounds such as nimbin, nimbidin,nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol. Nimbidin(the main active antibacterial ingredient), nimbin and nimbinin are stable compounds and found in considerable quantities [10-12]. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves of neem and were known to have antifungal and antibacterial activities [13]. Studies have confirmed their role as anti-inflammatory, ant arthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumour activities[14-17].

Sadabahar*(Catharanthus roseus)* also known as Madagascar periwinkle, rose periwinkle, or rosy periwinkle, native and endemic to Madagascaris a flowering plant belongs to the dogbane family Apocynaceae[18], [19].*Catharanthus roseus* is known for its microbial properties. It contains more than 70 different types of alkaloids (indole alkaloids) and chemotherapeutic agents[20].

Materials and Methods

Collections of samples –

Fresh leaves of Eucalyptus, Rose, Neem*(Azadirachta indica)* andSadabahar*(Catharanthus roseus)* were collected from Chandigarh, India region. Leaves were sorted, washed, dried at room temperature and milled into fine homogenous powder**.**

Extract preparation-

Eucalyptus, Neem & Sadabahar aqueous extract:

10 gram of dried powdered sample was soaked in 100ml of distilled water & left undisturbed for 24 hrs. After 24 hours, the suspension was shaken and this mixture was filtered through filter paper to obtain the aqueous extract. The filtrate was then stored in an air tight container[21].

Rose extract:

1000 grams of rose petals were sorted and washed. Petals were boiled in 500ml distilled water till the volume reduces to one third its initial volume. The extract was cooled, filtered and stored in an air tight container.

Preparation Of Hand Sanitizer-

Sterilized (autoclaved) extracts of eucalyptus and rose were mixed in equal volume (10 ml each) & 0.1ml of sterilized glycerin under aseptic condition in a sterilized container. The mixture was vortex in order to get homogenous solution.

Screening of Antimicrobial Activity

Evaluation of antimicrobial activity of aqueous Eucalyptus, Neem & Sadabahar extract was done by various methods; "Finger Imprint method", "Agar well diffusion method" and "Studying the effect of plant extract on micro flora".

[1] **Finger Imprint method:** Under aseptic conditions, sterilized nutrient agar was poured into sterile petri plates & allowed to solidify. Under working condition (outside of the laminar hood); For control - Four fingers (thumb not used) were gently but firmly pressed on the surface of solidified plates. The plate was labeled & incubated at 37° C for 24 hours. Autoclaved eucalyptus, neem & sadabahar extract were poured the in beakers. Eucalyptus extract was applied on the same 4 fingers & imprinted on the surface of solidified plates. The plate was labeled as 0 minute & incubated at 37° C for 24 hours. Wait for 2 minutes, imprint the plate as above. The plate was labeled as 2 minutes & incubated at 37° C for 24 hours. Similarly plates for 5 min, 10 min & 15 minutes were prepared, labeled& incubated at 37° C for 24 hours. After incubation, they're observed & using a colony counter count the number of colonies on each plate were counted. Similarly, neem and sadabahar extracts were evaluated**.[24]**

[2] **Agar well diffusion method:** Under aseptic conditions, sterilized Nutrient agar was poured into sterile petri plates & allowed to solidify. 0.1ml of culture was evenly spreaded on the surface of agar plates. Using a cork borer or a tip, a hole having a diameter of 6 to 8mm was punched. 0.1ml of extract of eucalyptus, neem & Sadabaharwas poured in each well. Plates were labeled and incubated at 37°C for 24 hours. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested therefore clear zone or zone of inhibition were observed**[21- 23].**

[3] **Studying the effect of plant extract on micro flora:**For control; Nutrient agar plate was placed in laminar hood (without any disinfection) for 15 minutes, labeled and incubated at 37°C for 24 hours. Then the laminar hood surface was then cleaned or disinfected with the aqueous eucalyptus, neem & sadabahar extract individually . Nutrient agar plates were placed in laminar hood for 15 minutes, labeled and incubated at 37°C for 24 hours. After incubation, the plates were observed for growth.

Since the results of eucalyptus extracts were most promising, eucalyptus extract was used for preparation of hand sanitizer. Antimicrobial activity of hand sanitizer was observed by Finger Imprint technique.

[4] **Finger Imprint method for hand sanitizer:** Under aseptic conditions, solidified nutrient agar plates were prepared. Under working condition (outside of the laminar hood); For control - Four fingers(thumb not used) were gently but firmly pressed on the surface of solidified plates. The plate was labeled & incubated at 37° C for 24 hours. Autoclaved hand sanitizer was applied on 4 fingers & imprinted on the surface of solidified plates. The plate was labeled as 0 minute & incubated at 37° C for 24 hours. Wait for 2 minutes, imprint the plate as above. The plate was labeled as 2 minutes & incubated at 37° C for 24 hours. Similarly plates for 5 min, 10 min & 15 minutes were prepared, labeled & incubated at 37° C for 24 hours. After incubation, they're observed & using a colony counter count the number of colonies on each plate were counted.**[24]**

Results

Method I: Reduction in microbial count after the use of aqueous extracts

The study elucidates the reduction in microbial count of hands after application of the aqueous extracts, Table 1 depicts the CFU per plate at various time intervals. It becomes lucid that the efficiency of eucalyptus was highest, followed by Sadabhar and Neem extracts, with an activation time of 5 minutes,10 minutes and 10

minutes respectively. The difference in the CFU count and activation time was quite significant among Eucalyptus (Figure 1 to 5) and the other two extracts.

Table1- Finger Imprint method

S.No	Time of exposure	Eucalyptus extract No of colonies obtained per plate	Neem extract No of colonies obtained per plate	Sadabahar extract No of colonies obtained per plate
1.	Control (No extract)	450	400	420
2.	0 minute	380	395	400
3.	2 minutes	167	370	340
4.	5 minutes	85	310	300
5.	10 minutes	51	278	263
6.	15 minutes	47	209	201

Figure 1: 0 Minute Figure 2: 2 Minutes Figure 3: 5 Minutes Figure 4: 10 Minutes Figure 5: 15 Minutes

Figure 1 to 5: Nutrient Agar Plates Containing Eucalyptus Extract

Method II: Antimicrobial efficacy of aqueous extract by agar well diffusion method

Zone of inhibitions (Table 2)(Figure 6) were observed in the nutrient agar plate on addition of aqueous extracts, the zone of clearance of 30mm, 28mm and 20 mm were observed for Sadabahar *(Catharanthu sroseus)*, Eucalyptus and Neem *(Azadirachta indica*) respectively. The extracts showed notable level of efficiency against microbial growth.

Table2-Method 2- Inhibition zone of aqueous extract of Neem, Eucalyptus and Sadabahar extract

Plant Extract Taken	Eucalyptus Extract	Neem Extract	Sadabahar Extract
Zone Of Inhibition On Nutrient Agar Plate (Aqueous Extract Taken)	28 Mm	20 Mm	30 Mm

Method III: The effect of aqueous extracts on air micro flora contamination

It becomes vivid that, no or negligible growth was observed on the plates obtained after their respective exposure of 15 minutes to the environment of laminar flow which was sanitized with Eucalyptus extract (fig 7.), Neem extract (fig8.), and Sadabahar extract (fig 9.)

Method 3- The effect of aqueous extracts on air micro flora contamination

Figure 7: Effect of Eucalyptus extract (aqueous) on air micro flora contamination

Figure 8: Effect of Neem extract (aqueous) on air micro flora contamination

Figure 9: Effect of Periwinkle or Sadabahar extract (aqueous) on air micro flora contamination.

Table 3: Total Plate Count of Agar plate for hand sanitizer using Finger Imprint technique

S.No.	Time of exposure	No of colonies obtained per plate
1.	Control(No extract)	350
2.	0 minute	270
3.	2 minutes	140
4.	5 minutes	60
5.	10 minutes	45
6.	15 minutes	30

Method IV: Antimicrobial evaluation of hand sanitizer by finger imprint technique

The Table 3 depicts the reduction in the colony forming units after usage of the hand sanitizer. The microbial count plunged from 270 to 30 in the time span of 15 minutes. The hand sanitizer developed was efficient in elimination of microbes.

Conclusion

Due to recent awareness regarding hygiene and sanitation, the usage of alcohol based sanitizers has elevated, irrespective of the side effects, therefore it is important to develop the eco friendly substitutes.

This study confirmed the antimicrobial proprieties of aqueous extract extracted from the leaves of some plants like Eucalyptus, Sadabahar*(Catharanthus roseus)* and Neem *(Azadirachta indica*). The aqueous extract has more economical for commercial production than essential oil. Eucalyptus extract showed prominent antimicrobial properties therefore along with rose extract and glycerin was used to develop the hand sanitizer. The results showed that hand sanitizer effectively reduced bacterial load on hands to a varying degree.

By amalgamating, all the results it can be stated that herbal extracts especially Eucalyptus has a lot of potential that is still left to be explored and can be exploited to develop ecological substitutes of various chemical products.

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