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**ANTI-MICROBIAL ACTIVITY OF SILVER NANO-PARTICLES SYNTHESISED  
USING HERBAL FORMULATION**

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**ABSTRACT**

**Aim:** To assess the antimicrobial property, synthesis and characterization of Silver nano particles using herbal formulations of lycopene, raspberry and green tea. **Materials and methods:** the synthesis of nanoparticles was done using lycopene (25%), raspberry (10%) and green tea (95% polyphenols) and 0.016 gms of silver nano-particles, characterization was done using transmission electron microscope (Zeiss 120 TEM), anti-microbial test using disc diffusion method in the most common 4 oral commensals. **Results:** The present assay shows that the highest area of inhibition is seen in *C.albicans* and the lowest inhibition seen in *Staphylococcus aureus* in comparison with the standard antibiotics seen, the shape of the nano particle obtained was spherical. **Conclusion:** From the above results it can be concluded that the combination of silver nano particles infused media has a better anti-microbial property, especially as an anti-fungal.

**Keywords: Lycopene, nanoparticle, antimicrobial**

## INTRODUCTION

Silver nano-particle has been well known as an effective anti-microbial material and has no side effects. This is due to their size and permeability into the cell membrane which can alter the structure of cell membrane and finally resulting the death of cell [1-3]. The effective of silver nanoparticles against gram-negative food borne bacteria that is *Escherichia coli*, *Salmonella enteritidis* and *Klebsiella pneumoneae* including the shorter period of killing or destruction activity [4-6]. The assessment of the antimicrobial property of silver-nanoparticles can be done only after the evaluation of its size, shape, surface area and the distribution, they have been used in the fields of diagnosis, anti-cancer management [7-9]. The synthesis of silver nanoparticles are commonly done using chemical method, other methods include physical and biological methods, chemical method is cost-effective and has more yield of particles [10-12]. The use of lycopene is because of its high anti-oxidant and microbial activity along with the presence of raspberry and green tea is an highly solvable and effective compound against most common oral commensals such as *E.coli*, *S.aureus*, *S.mutans*, with the presence of silver nanoparticle, the solubility and permeability is increased thus increasing its

effective as an disinfection and the sterilizing property [13-15]. The epicatechin gallate present in the green tea has a moderate potent of microbial activity inhibition, among the oral commensals the *S.aureus* and the beta lactam microbes are the most susceptible [17]. Raspberry extracts have been shown to be effective against the about 16 types of microbes including the most common oral commensal *S.aureus*, and has strong inhibitory activity

## MATERIALS AND METHODS

### *Synthesis and characterization of silver nanoparticles infused herbal extract:*

Materials used includes raspberry (25%), lycopene (10%), green tea (95% polyphenols) and 0.016 gm silver nano particles, concentration of extract by boiling at 50 degree Celsius for 30min, centrifuging is done at 8000RPM for 10 mins using refrigerated Centrifuging method, finally separation of concentrated extracts, characterization of silver nano particles infused herbal formulation was done using Transmission electron microscope (Zeiss libra 120 TEM) (Figure 3).

### *Anti-Microbial testing:*

The antibiotic testing followed in the current study is agar diffusion method or kirby-bauer method. Four petri dishes containing agar

media are then subjected to the application of 4 most common oral commensals such as *S.aureus*, *E.faecalis*, *C.albicans*, *S.mutans*. The process by which these organisms are taken is using a sterile swab from the respective broth culture in an aseptic method, they are subjected into the wells containing the antibiotics (silver nanoparticles infused lycopene, raspberry, green tea) in the quantity of 25µL, 50µL and 100µL along with a control antibiotic (such as amoxicillin) in the four respective petri dishes. An area of demarcation is formed around the wells where the antibiotics are subjected to. The extent from the edge of the wells to the area of demarcation is measured which is known as the zone of inhibition, this indicates the effective inhibition or stoppage of bacterial growth by the antimicrobial that is subjected (Figure 1).

## RESULTS

The results of this study show that the combination of lycopene, raspberry, green tea and silver nanoparticle formulation have better antimicrobial efficiency, the antimicrobial efficiency of the herbal formulation when compared with control antibiotic, had good levels of effective zone of inhibition. The present assay shows that the highest area of inhibition is seen in *C.albicans* with an area of 35mm in

100µl concentration and the lowest inhibition seen in *Staphylococcus aureus* (9mm at 25µl concentration) in comparison with the standard antibiotics seen, the shape of the nano particle obtained was spherical, with sizes differing from 4 to 50nm, AgNP's peak absorbance occurs at 490 nm with high absorbance.

## DISCUSSION

Study done by Faizan *et al.*, (2019) using silver nanoparticles infused *MK (Murraya koenigii)* has revealed the inhibition zone of diameters 21mm and 19 mm against *MRSA (methicillin resistant Staphylococcus aureus)*, but the current study the *S.aureus* variant has the lowest inhibition percentage of 9mm in the dosage of 25µl and the highest being 25 mm at concentration of 100 µl [18-20] (Figure 2), while other studies by Stefan *et al* (2020) has shown the antimicrobial activity of specific oral commensals such as *P.gingivalis* and *A.naeshlundii*, but the highest inhibitory zone in *P.gingivalis* (3 mm) but while in the current study the minimum inhibitory concentration was 9 mm which is against *S.aureus*, while in the respected article the inhibition area was Nil against *S.aureus* which proves the effectiveness of the current herbal formulation infused with nanoparticles [21, 22]. Studies showing the effectiveness of

green by Wanda.C (2014) has shown the inhibitory of EGCG present in green tea showing lytic activity against gram negative oral commensals, with *E.coli* being the most susceptible to destruction, other bacteria of interest that also undergoes lysis include *H.pylori* and *S.aureus* [22] (Table 1). Studies on Raspberry extracts by Octavio et al (2012) has proven the effectiveness of *S.mutans* and *P.gingivalis*, an area of inhibition at the concentration of 350 µg/ml 23.9mm, and the highest inhibition

concentration of 28.6mm seen against *P.gingivalis* species, the process of immunity by this particular black raspberry species is due to its increase strain reduction against oral bacteria especially *F.nucleatum* (showing 36mm at the dose of 1400 µg/ml), which in the present study the maximum area of inhibition of 35mm is seen against *Candida albicans* which makes the present combination the best anti-fungal, followed by 24mm inhibition against *Streptococcus mutans* [23-25].

Table 1: represents the comparison of zone of inhibition between antibiotic and lycopene, raspberry, green tea and silver nano-particles formulation

| Species               | Control Antibiotic- Zone of Inhibition | Herbal Formulation-Zone of Inhibition |
|-----------------------|--|---------------------------------------|
| Staphylococcus Aureus | 12mm                                   | 23mm                                  |
| Streptococcus mutans  | 22mm                                   | 36mm                                  |
| Enterococcus faecalis | 20mm                                   | 38mm                                  |
| Candida albicans      | 35mm                                   | 20mm                                  |

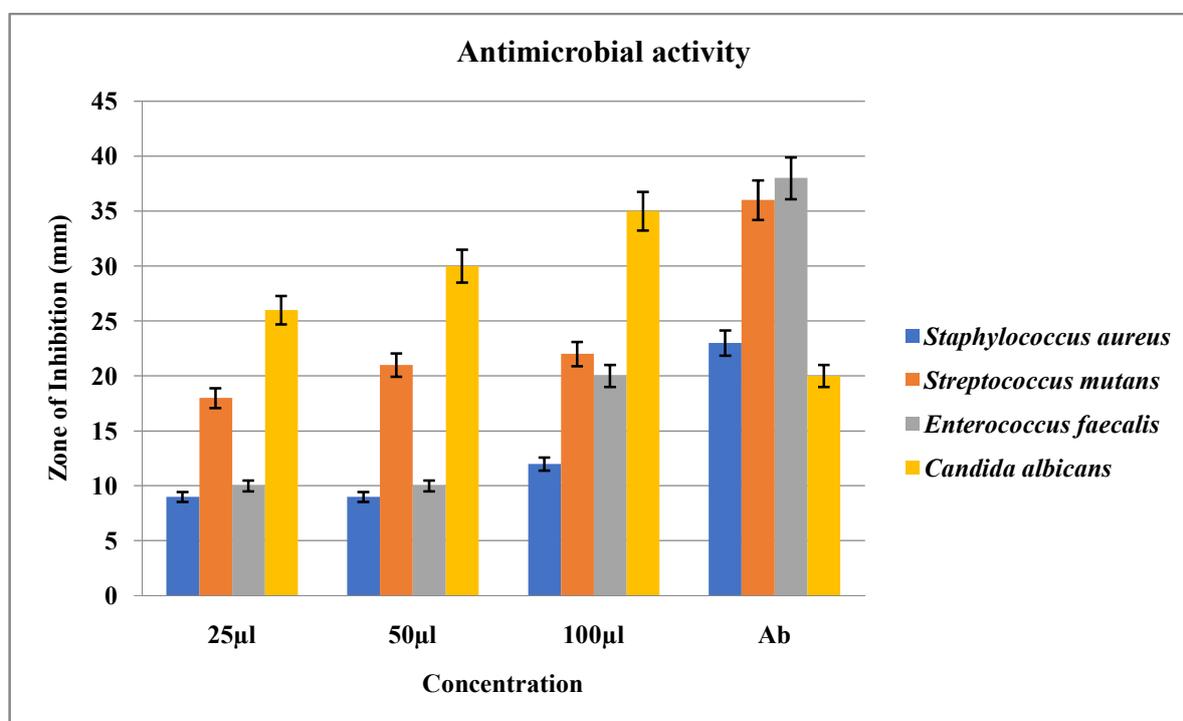


Figure 1: Anti-microbial zone of inhibition seen among the various

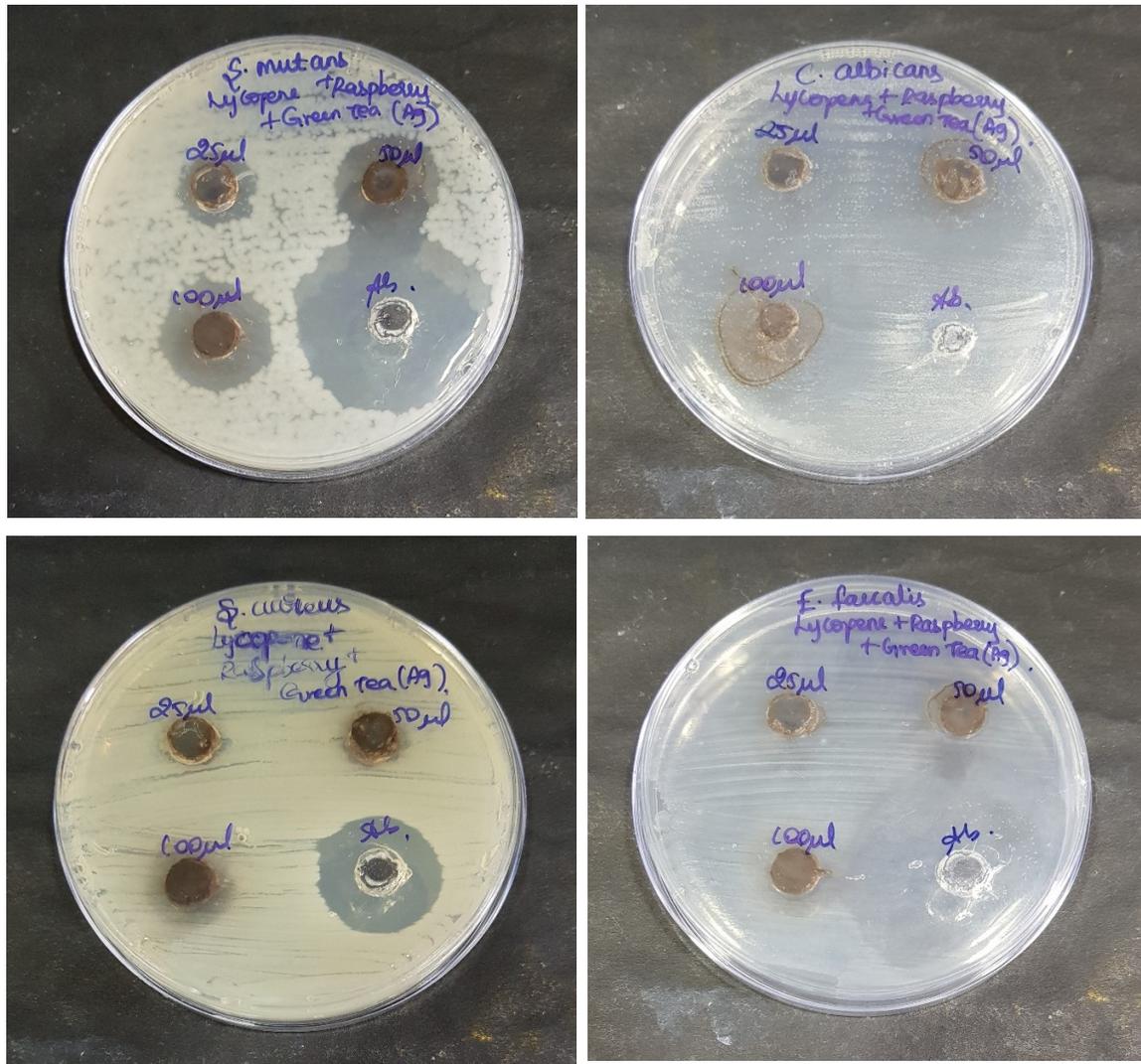


Figure 2: shows agar media containing various microbial compounds and the respective area of inhibition

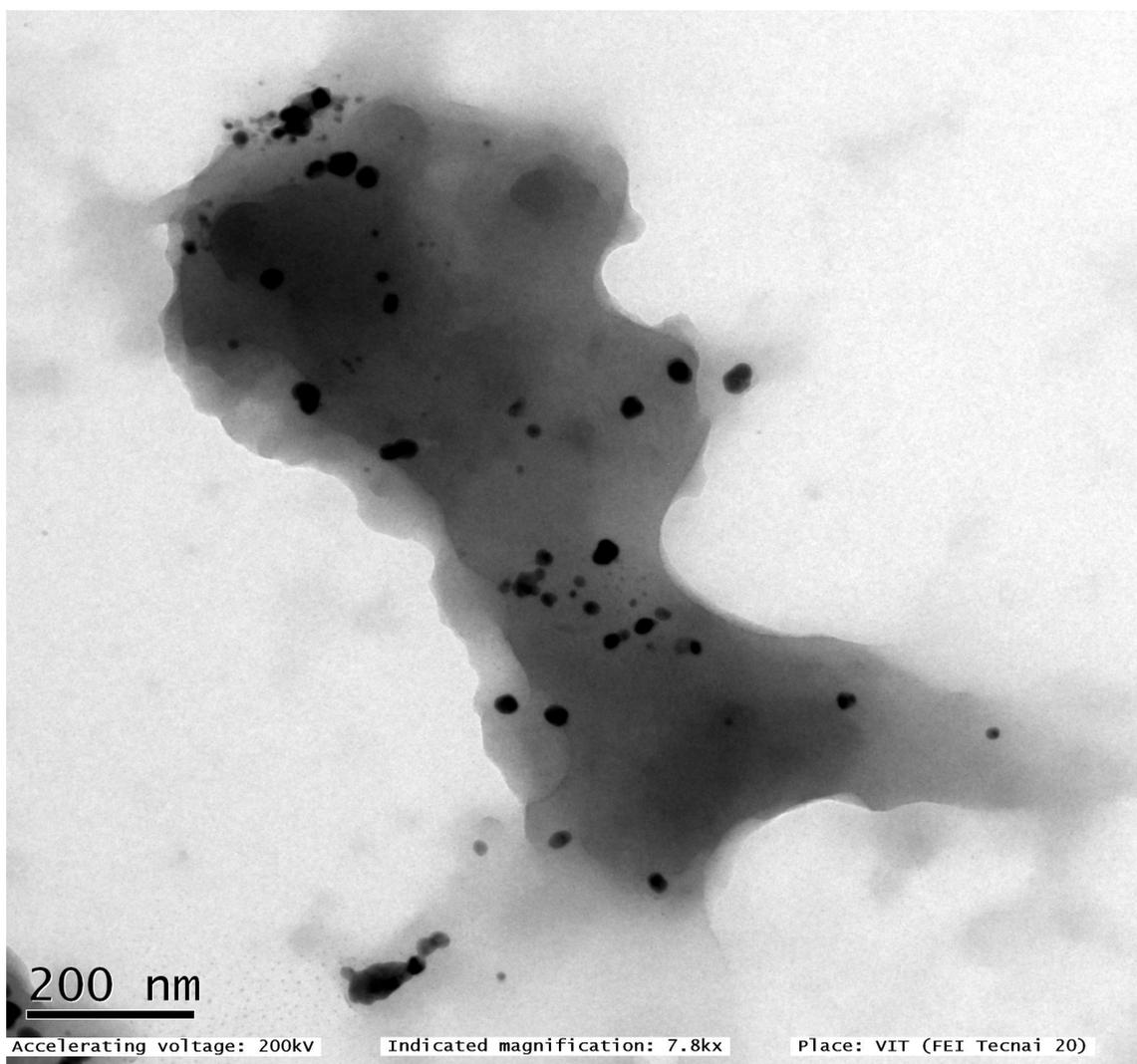


Figure 3: Characterization of nano-particles done under Transmission electron microscope (TEM) at 200nm

## CONCLUSION

The present study concluded that lycopene, raspberry, green tea and silver nanoparticles combination has a significant anti-microbial property, among the 4 most common oral commensals tested, based on the microbial inhibition, it is highly effective anti-fungal and anti-bacterial formulation.

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