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Unveiling the Therapeutic Potential of Annona muricata and Psidium guajava through Nanotechnology: A Focus on Anti-inflammatory, Antioxidant, and Antimicrobial Activities.

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Abstract:
Nanoparticles synthesized using herbal incorporation leverage the natural compounds found in plants for their production. This method is often more environmentally friendly compared to conventional chemical synthesis. Nanoparticles produced using plant extracts are often more compatible with biological systems. Different plants can impart unique properties to the nanoparticles, such as antioxidant, antimicrobial, or anti-inflammatory activities. Incorporating bioactive compounds from soursop (Annona muricata) and guava (Psidium guajava) into nanoparticles offers several potential benefits, particularly in the fields of food science, medicine, and cosmetics. These tropical fruits are rich in antioxidants, vitamins, and other bioactive compounds, and their encapsulation into nanoparticles can enhance the stability, bioavailability, and targeted delivery of these beneficial compounds. Our study one among the fewer studies done with herbal and nanoparticle combination made an attempt to study their antioxidant, antimicrobial and anti-inflammatory activity.
Key Words: Silver nanoparticles, herbals, bioactive compounds.

Introduction:

“Nanotechnology's broad impact across diverse research domains, highlights its interdisciplinary nature. Nanoparticles, especially those crafted via environmentally friendly methods, afford precise control over material characteristics at the nanoscale, promising significant applications in pharmaceuticals and nutraceuticals. Green production of nanoparticles emphasizes the importance of environmentally sustainable synthesis approaches, which mitigate the generation of harmful byproducts often associated with traditional methods [1]. Psidium guajava (Guava) and Annona muricata (Soursop) are introduced as tropical fruits renowned for their diverse medicinal properties, including antimicrobial, anti-inflammatory, antioxidant, and anticancer attributes [2]. Guava (Psidium guajava) is a plant of the family “Myrtaceae”. These compounds exhibit antioxidant properties, helping to scavenge free radicals and mitigate oxidative stress, thus potentially reducing the risk of chronic diseases [3]. Psidium Guajava (P.guajava) and Annona Muricata (A.muricata) contains compounds such as flavonoids, tannins, and essential oils that exhibit antimicrobial activity which combat against oral pathogens,thus reducing the dental infections. It is also an excellent source of vitamin C, which supports gum health by promoting collagen synthesis. Psidium Guajava attributed to its polyphenolic compounds like quercetin and kaempferol, acts as anti-inflammatory herbal which reduce inflammation associated with periodontal diseases. Annona Muricata serves as an antioxidant which help neutralize free radicals and protect oral tissues from oxidative damage. Their rich history of traditional medicinal usage provides a compelling foundation for exploring their potential in nanoparticle synthesis.

MATERIALS & METHODS:

The procedures involved in this study conducted by the authors were approved by the Ethics Committee of Dr. M. G. R. Educational and Research Institute. Annona Muricata and Psidium Guajava leaves were procured, dried and powdered. The obtained powder is then preserved in an air sealed container. Following which one gram of Annona Muricata and Psidium Guajava leaf powder is diluted with 40 ml of distilled water and boiled for 20 min in a stirrer which allows uniform dispersion of herbal powder. The herbal extract was filtered using Whatman No.1 filter paper and left undisturbed for 20 min. Subsequently, the plant extract was transferred to a sealed container, refrigerated overnight, and utilized for green synthesis.

Silver nitrate solution preparation:

To prepare Silver Nitrate solution, 0.06gm of Silver Nitrate was weighed and dissolved in 60ml of distilled water. This solution was then mixed with 40 ml of filtered plant extract and stirred in a magnetic

stirrer for 1 hour. The mixture was subsequently placed in a shaker to facilitate thorough particle intermixing leading to green synthesis. The reduction of silver nitrate to silver nanoparticles (AgNPs) was periodically monitored using UV spectrophotometer [3,4]and colour change was observed visually. The synthesized AgNP solution was centrifuged at 8000 rpm for 10 minutes using a Lark refrigerated centrifuge, and the resulting pellet was collected. The pellet was washed twice with distilled water to ensure purity. Finally, the purified pellet was dried at 100–150°C for 24 hours to obtain nanoparticle powder, which was then transferred to an airtight Eppendorf tube.

ASSESMENT OF ANTI-INFLAMMATORY ACTIVITY:

Anti-inflammatory activity using Albumin Denaturation Assay:

Test Group:

In five separate test tubes, 10, 20, 30, 40, and 50 µL of nanoparticles were measured out. To each test tube, 2 mL of 1% bovine serum albumin (BSA) solution was added. Additionally, 390, 380, 370, 360, and 350 µL of distilled water were added to the test tubes containing 10, 20, 30, 40, and 50 µL of nanoparticles, respectively.

Standard group:

Volumes of 10, 20, 30, 40, and 50 µL of diclofenac sodium were measured and added to five separate test tubes. Each test tube was supplemented with 2 mL of 1% BSA solution. The samples were first incubated at room temperature for 10 minutes, followed by incubation in a water bath at 55°C for approximately 10 minutes. Absorbance readings were then recorded using a UV spectrophotometer at a wavelength of 660 nm. Percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

Egg Albumin Denaturation Assay:

For the egg albumin denaturation assay, 0.2 mL of fresh egg albumin was combined with 2.8 mL of phosphate buffer. Various concentrations (10-50 µg/mL) of Annona muricata and Psidium guajava silver nanoparticles were added to the mixture. The pH was adjusted to 6.3, and the solution was allowed to stand at room temperature for 10 minutes. It was then incubated in a water bath at 55°C for 30 minutes. Diclofenac sodium served as the standard control. Finally, the absorbance of the samples was recorded using a spectrophotometer at 660 nm. Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Membrane Stabilization Assay:

The in vitro membrane stabilization assay is a commonly employed method for assessing the membrane-stabilizing potential of both natural and synthetic compounds. This technique evaluates a compound's ability to protect cell membranes from disruption and prevent the release of intracellular components. To prepare the RBC suspension, fresh human blood is collected into a sterile tube containing an anticoagulant. The blood is centrifuged at 1000 g for 10 minutes at room temperature to separate the red blood cells (RBCs) from other components. The supernatant is discarded, and the RBCs are washed three times with phosphate-buffered saline (PBS). Finally, the washed RBCs are resuspended in Tris-HCl buffer to achieve a 10% (v/v) RBC suspension.

Assay procedure:
Transfer 1 mL of the prepared RBC suspension into each centrifuge tube. Add varying concentrations of A. muricata and P. guajava silver nanoparticles to the reaction mixtures. Adjust the pH of the mixtures to 6.3. Mix the contents gently and incubate the tubes at 37°C for 30 minutes. After incubation, centrifuge the tubes at 1000 g for 10 minutes at room temperature to pellet the RBCs. Finally, measure the absorbance of the supernatant at 540 nm using a UV-Vis spectrophotometer. Calculate the percentage inhibition of hemolysis using the following formula:

% inhibition = [(OD control – OD sample) / OD control] x 100

Here, OD control refers to the absorbance of the RBC suspension without the addition of the test compound(s), while OD sample represents the absorbance of the RBC suspension containing the test compound(s).

EVALUATION OF ANTI-OXIDANT ACTIVITY:
Test Group:
A series of test tubes were prepared by adding 10, 20, 30, 40, and 50 µL of the nanoparticle solution to five separate tubes. To each test tube, 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was added. Subsequently, 1990, 1980, 1970, 1960, and 1950 µL of 50% methanol solution were added to the test tubes containing 10, 20, 30, 40, and 50 µL of nanoparticles, respectively.

Standard group:
Ascorbic acid was used as the standard. The test tubes were incubated for 20 minutes in a dark cupboard to prevent light interference. After incubation, the absorbance was measured at 517 nm using a UV spectrophotometer. The percentage inhibition was then calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

H2O2 scavenging activity

The hydrogen peroxide (H_2O_2) scavenging activity of A. muricata and P. guajava silver nanoparticles was evaluated. A 40 mM solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). Different concentrations of A. muricata and P. guajava silver nanoparticles, along with standard ascorbic acid (10–50 µg/mL), were added to 0.6 mL of the H_2O_2 solution. The mixtures were incubated for 10 minutes at room temperature. Following incubation, the absorbance of the test samples was measured at 230 nm using a spectrophotometer. The percentage of inhibition activity was calculated using the following formula:

Percentage of inhibition = (OD control – OD sample) / OD control x 100.

ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of green-synthesized A. muricata and P. guajava silver nanoparticles was assessed using the agar well diffusion method. Mueller-Hinton agar plates were prepared and sterilized in an autoclave at 121°C for 15–20 minutes. The sterilized medium was poured into sterile Petri plates and allowed to cool to room temperature.

Bacterial suspensions (Streptococcus mutans, Lactobacillus species, Staphylococcus aureus, Candida albicans, and E. coli) were evenly spread over the agar surface using sterile cotton swabs. Wells of 9 mm diameter were created in the agar using a sterile polystyrene tip. Different concentrations of A. muricata and P. guajava silver nanoparticles (25 µg, 50 µg, and 100 µg) were added to the wells. A. muricata and P. guajava extracts were used as standards.

The plates were incubated at 37°C for 24 hours for bacterial cultures and 48 hours for fungal cultures. Antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells. The diameter of the zones was measured in millimeters using a ruler, and the results were recorded.

RESULTS:

The study has evaluated the anti-oxidant, anti-inflammatory property at various concentrations. When analysing the results, herbal incorporation of nanoparticles was almost in line with that of standard. Figure 1 depicts the anti-inflammatory property of silver nanoparticles, Psidium guajava and Annona Muricata using albumin denaturation assay. The assay was done by utilizing various concentration of prepared extract and compared with standard values. It was found that the values of anti-inflammatory property of nanoparticles was found to be similar to that of standard at various concentrations(10,20,30,40,50 µg/ml).Percentage of inhibition of silver nanoparticles was found to be 41% at 10µg/ml, 52% at 20 µg/ml, 65% at 30µg/ml, 73% at 40µg/ml and highest 80% at 50µg/ml (Table 1)

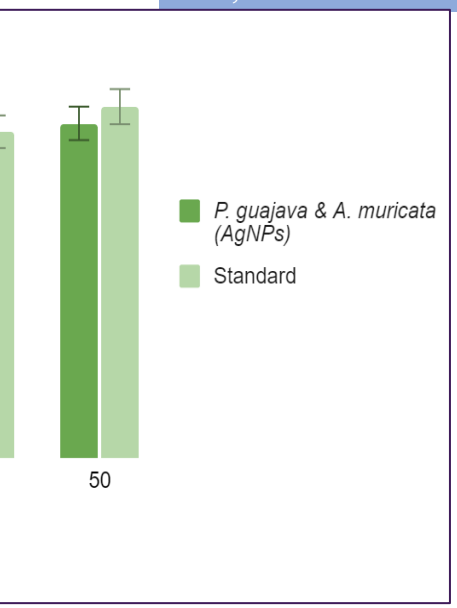

Figure 1-Bovine Serum Assay

BSA(µg/ml)	**10**	**20**	**30**	**40**	**50**
P. guajava & A. muricata (AgNPs)	41	52	65	73	80
Standard	47	60	72	78	84

Table 1

Figure 2 and Table 2 depicts the anti-inflammatory activity of Psidium guajava and Annona Muricata using egg albumin denaturation assay (EA Assay). The results revealed highest percentage of inhibition 77% at the concentration of 50µg/ml which is nearer to that of standard 81%.

Membrane stabilization assay (MSA) was done to confirm the anti-inflammatory property of Psidium guajava and Annona Muricata incorporated silver nanoparticles. Similar results were obtained as in albumin denaturation assay and egg albumin denaturation assay. The percentage of inhibition was (53%, 64%,72%,77%,84%) at various concentration of 10,20,30,40, 50µg/ml (Figure 3 and Table 3)

Antioxidant activity Psidium guajava and Annona Muricata incorporated silver nanoparticles was assesed using DPPH assay (Figure 4 and Table 4) and H2O2 assay (Figure 5 and Table 5). The results revealed that standard showed higher antioxidant activity than prepared nanoparticles. However at various concentration (10,20,30,40,50 µg/ml), antioxidant activity of prepared nanoparticles was similar to that of standard.

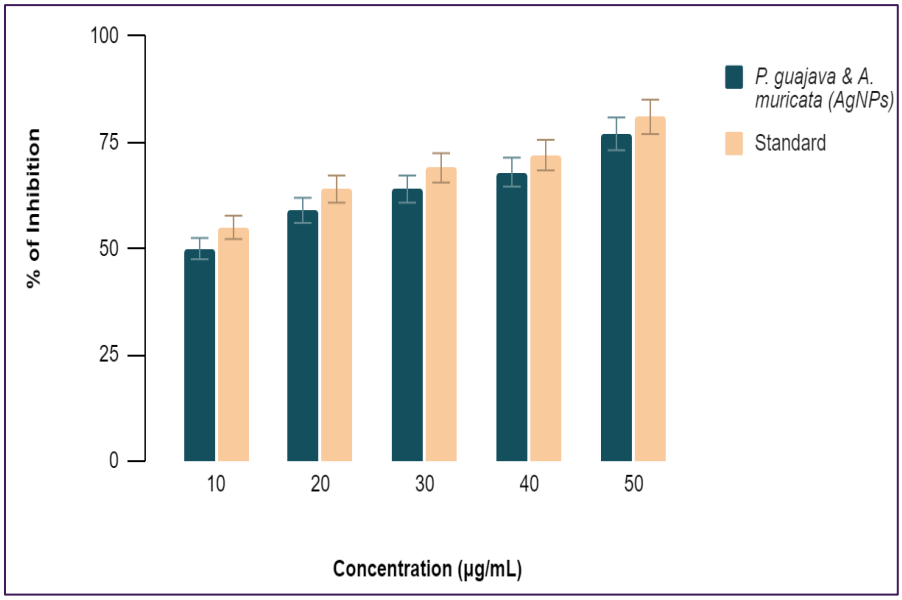

Figure 2- Egg Albumin Assay

EA (µg/ml)	**10**	**20**	**30**	**40**	**50**
P. guajava & A. muricata (AgNPs)	50	59	64	68	77
Standard	55	64	69	72	81

Table 2

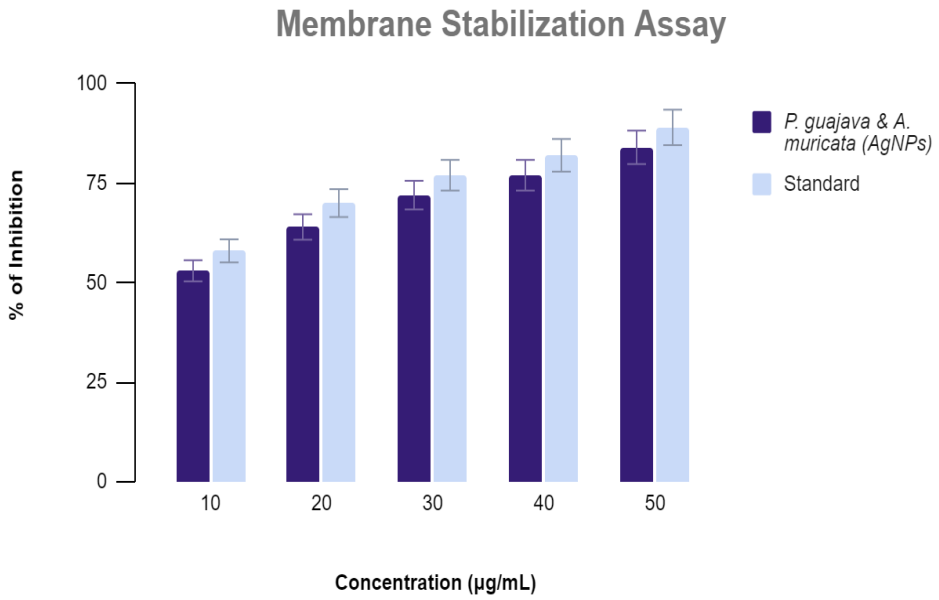

Figure 3- Membrane Denaturation Assay

MSA (µg/ml)	**10**	**20**	**30**	**40**	**50**
P. guajava & A. muricata (AgNPs)	53	64	72	77	84
Standard	58	70	77	82	89

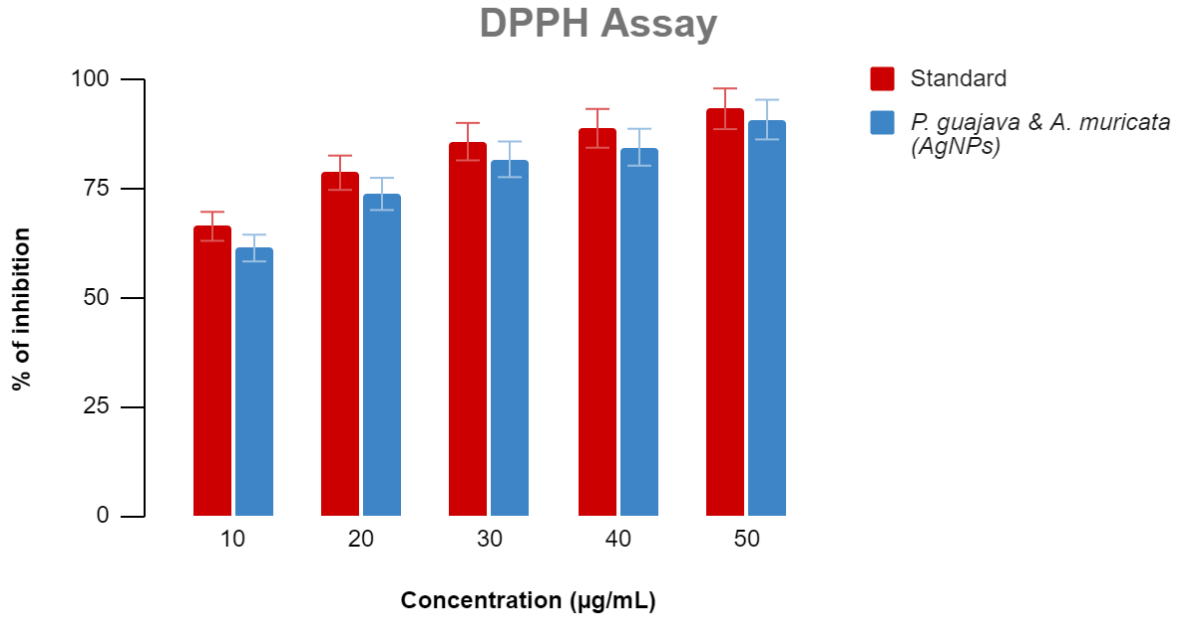

Figure 4- DPPH ASSAY

DPPH (µg/ml)	Standard	P. guajava & A. muricata (AgNPs)
10	66.25	61.28
20	78.52	73.67
30	85.63	81.59
40	88.68	84.36
50	93.15	90.68

Table 4

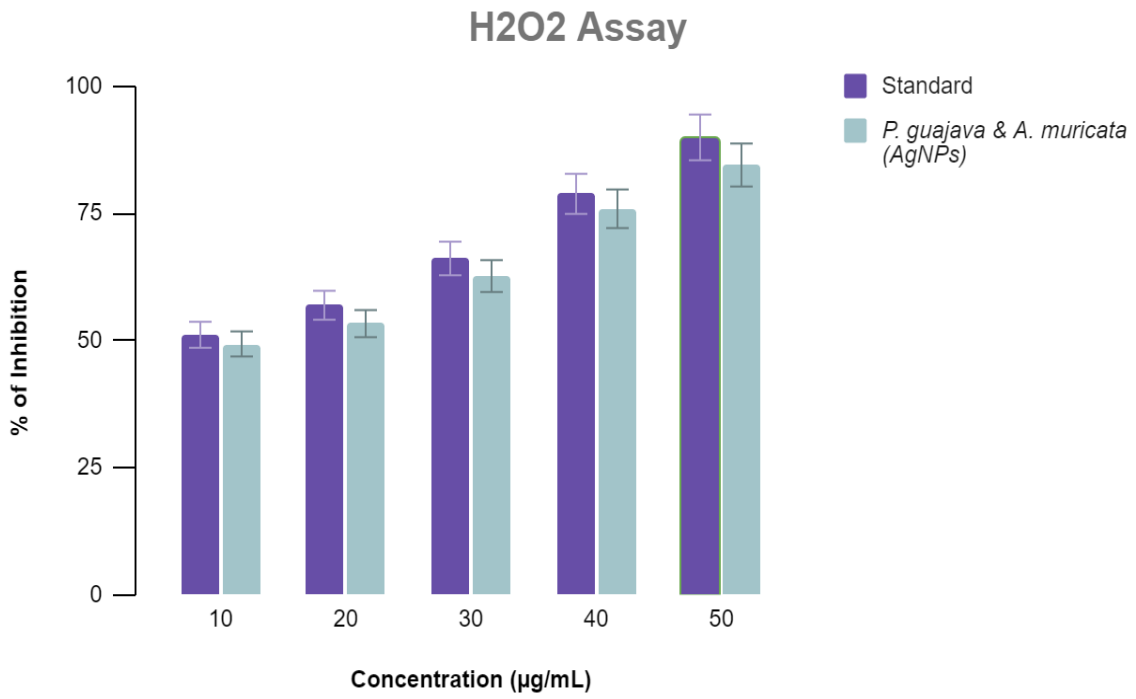

Figure 5- H2O2 ASSAY

H2O2 (µg/ml)	Standard	P. guajava & A. muricata (AgNPs)
10	51.1	49.3
20	56.9	53.29
30	66.1	62.64
40	78.8	75.87
50	89.9	84.47

Table 5

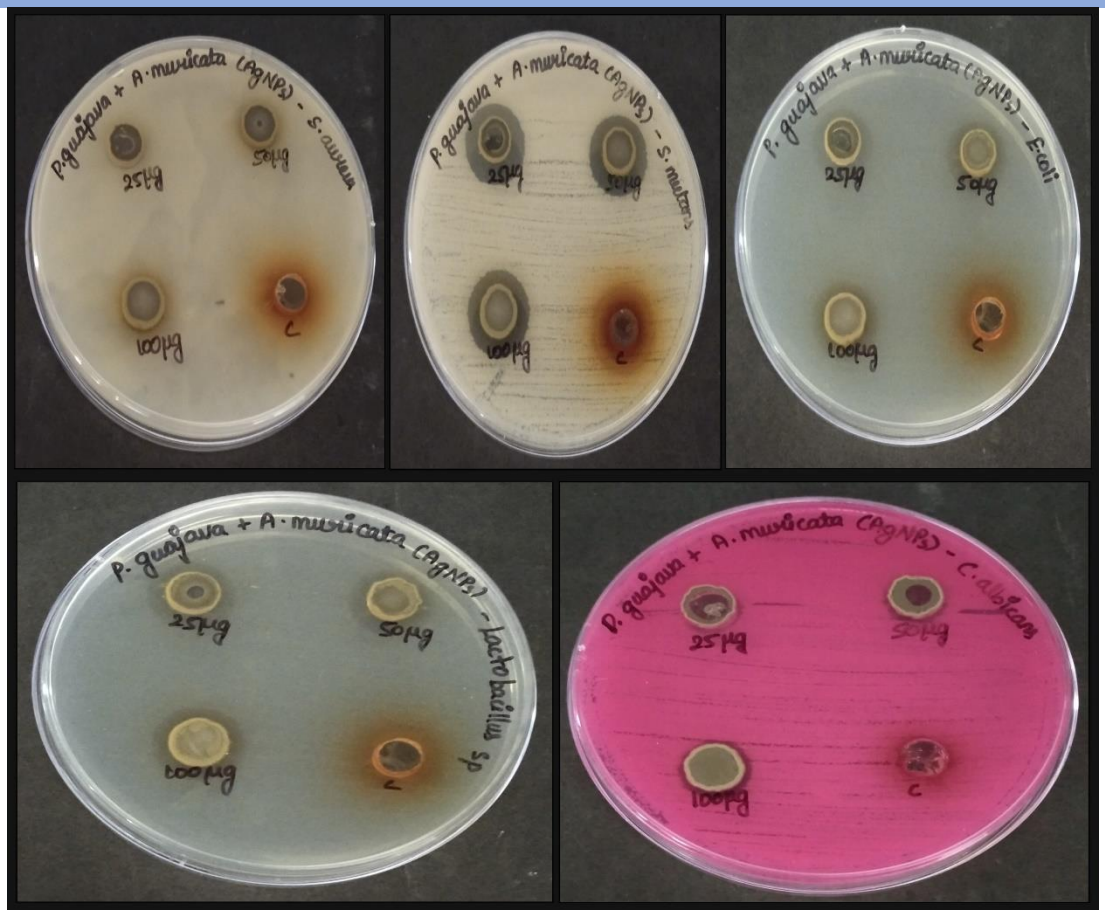

Figure 6 - Zone of Inhibition of P.guajava and A.muricata incorporated AgNPs at various concentration (µg/ml) against Staphylococcus aureus (A), Streptococcus mutans(B), E.coli (C), Lactobacillus Species(D) and Candida albicans(E).

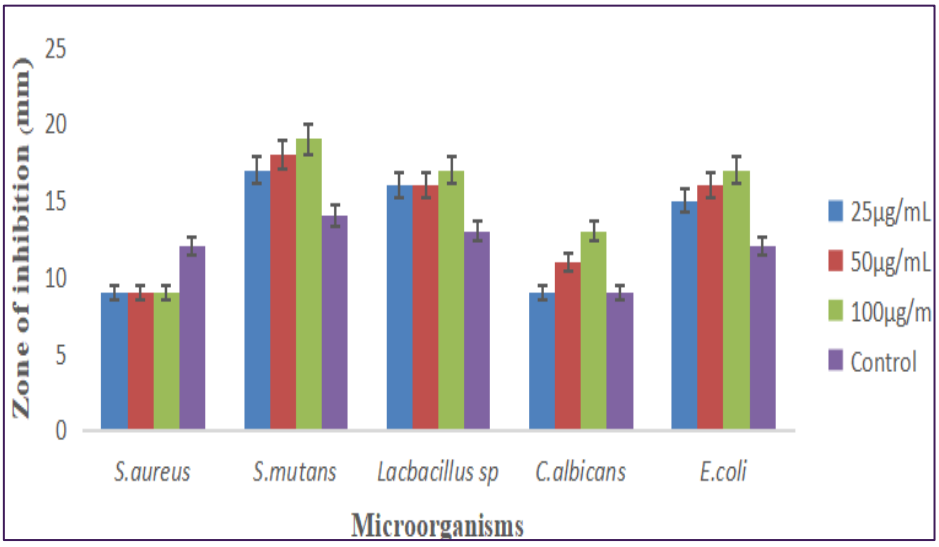

Figure 7

The antimicrobial activity of herbal incorporated silver nanoparticles was evaluated using agar well diffusion method (Figure 6). The herbal incorporated nanoparticles exhibited zone of inhibition of 20mm with a dilution of 100µg/mL against Streptococcus mutans while 15mm was zone of inhibition for the control. The nanoparticles developed zone of inhibition of 14mm with a dilution of 100µg/mL of herbal nanoparticles against Candida albicans while 9mm was the zone of inhibition for the control.

Zone of inhibition of herbal nanoparticles at 100µg/mL against Lactobacillus species and E.coli was 18mm against control which shows the measurement of 14mm. The herbal nanoparticles exhibited zone of inhibition of 15mm,16mm and 18mm against Lactobacillus and E.coli species at three different concentration (25,50,100µg/mL) indicating almost similar level of inhibition for these strains(Figure 7). In comparison, the fourth well which contained the control silver nanoparticles showed the higher zone of inhibition of 14mm and 15mm against Streptococcuss mutans and Lactobacillus species and lower area of inhibition of 9mm against Candida albicans at the same dilution concentration whereas 12mm zone of inhibition exhibited by the control in E.coli and Staphylococcus aureus strains.

DISCUSSION:

According to a report by the World Health Organization (WHO), about 80% of the population in the developing world relies on traditional plant extracts as antioxidants, antibacterial, antifungal, and antiviral agents. Ethnobotanic medicine, a field where parts of plants are used for medicinal purpose is evolving very rapidly [5]. The mode of action of soursop and guava leaves as a potent antimicrobial, antifungal and antioxidant has been studied and reviewed in various studies. Guava fruits and leaves are high-value-added plants with great utility as functional cosmetics and food materials.

Various studies have proved that nanoparticles such as silver, copper, and zinc oxide possess strong antibacterial properties that can be utilized in dentistry to minimize the risk of oral infections [6,7]. These nanoparticles are integrated into products like toothpaste, mouthwashes, and dental coatings to inhibit bacterial growth and prevent plaque buildup. Additionally, the antibacterial and anti-inflammatory effects of guava leaves (Psidium Guajava) and soursop (Annona muricatta) can alleviate toothaches. Joana . L. et al [8] in 2019 compared the antibacterial effect of different concentrations of Annona muricata L. extracts on Streptococcus mutans and concluded that Annona muricata L. has more antibacterial activity [9,10].

Guava (Psidium guajava) is a dicotyledonous plant of the Myrtaceae family. It is widely distributed in tropical and subtropical regions of America. Guava can be said to be an “alternative crop that can cope with environmental changes” caused by global warming in terms of its cultivation environment and physiological characteristics. In a study done by Hyonam Park et al [11] in 2024, it was concluded that guava leaf extract concentrate has an antioxidant effect due to the radical scavenging activity of phenolic compounds such as sesquiterpenes. It also has a high content of polyphenols and flavonoids, which are indicators of antioxidant activity and inhibits collagenase activity.

The leaf of the Annona muricata (Soursop) has been traditionally considered “ The Cancer Killer ” in conventional medicine [7,8]. Chaterina Diyah Nanik et al9 in 2023, done a study using soursop leaf effervescents on denture base resin and concluded that Annona muricata can be used as denture cleanser and did not affect the transversal strength of the material when immersed in variuos concentration of extract Anonna muricata leaves effervescent [10,12]. Soursop is a pleasantly flavoured tropical fruit commonly consumed in Peru and is of great importance due to its health benefits.

Biologically these were achieved through acetogenins which are bioactive compounds known for their tumoricidal, antimalarial, antihelmintic, antiviral and antimicrobial effects [13,14]. Among the acetogenins, bullatacin is considered to be an effective tumoricidal agents [15,16]. However vast studies on incorporation of these magic bullet ethobiomedicine compounds with silver nanoparticles were less.

Hence in this study we have incorporated the herbal extracts of Annona muricata and Psidium guajava with silver nanoparticles and their antioxidant, antiinflammatory, antimicrobial activities are evaluated particularly for oral pathogens [17].

Our study, which used combination of these two herbals extracts (Annona muricata and Psidium guajava) incorporated with silver nanoparticles has reached positive results exhibiting almost similar anti-inflammatory and antioxidant activity to that of commercial available medicines. Our study had also evaluated the antioxidant and anti-inflammatory activity at various concentrations, however the concentrations are anectodal. We have also compared the silver nanoparticles which acts as control with herbal incorporated silver nanoparticles which exhibited improved antioxidant, antinflammatory and anti-microbial activity symbolizing the efficieny of these herbals. When various concentrations were analysed, our preparation showed better antioxidant and anti-inflammatory properties at concentrations of 50 µl. This paves way for further formulation of various herbal products using this nanoparticles which can be used in various fields of medicine and dentistry. Encapsulating these antioxidants in nanoparticles can enhance their stability and reactivity, improving their ability to scavenge free radicals and protect cells from damage. Nanoparticles can penetrate microbial cell walls more easily, delivering the active compounds directly to the site of infection. This increases the effectiveness of guava and soursop as natural antimicrobials [17]. They also exhibit synergistic effects when combined, enhancing their antimicrobial action against drug-resistant pathogens like E. coli, Streptococcus mutans, Staphylococcus aureus, and Candida albicans discussed in this study.

Limitations of the study is an exploratory study can be performed by increasing the herbal concentration of extracts. This study was performed to see incorporation of nanoparticles with herbals on oral microbial flora was worth tracking.

CONCLUSION:

Overall, the incorporation of herbal extracts in nanoparticle synthesis is a promising area of research that combines traditional knowledge with modern technology. Incorporating soursop and guava bioactives into green synthesized nanoparticles is a promising approach that can enhance their therapeutic and functional benefits. Thus,combining guava and soursop compounds within nanoparticles may produce a synergistic effect, enhancing the overall antioxidant and anti-inflammatory response.

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