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**COMPARATIVE ANTI-MICROBIAL ACTIVITY OF *VANASPATI MARITA*
YASHADA BHASMA AND GREEN SYNTHESIZED ZnO NANOPARTICLES
(GS ZnO NP) USING ALOE-VERA LEAF EXTRACT**

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ABSTRACT

Yashada bhasma, a traditional Ayurvedic metal-based preparation of zinc oxide nanoparticles used in wound care and diverse health conditions. Similarly in contemporary science, green-synthesized zinc oxide nanoparticles (GSZnO NP) are recognized for their antimicrobial activity. As both comprise zinc oxide nano particles, the present study aimed to compare their antimicrobial efficacy, employing *yashada bhasma* prepared via the *vanaspatimarita* (Calcination using herbal drug) method with *kumari swarasa* (aloe vera juice) as a *bhavana dravya*, and GSZnO NP synthesized using aloe vera aqueous extract. This study employed minimum inhibition concentration (MIC) assays and agar disc diffusion methods to compare the anti-microbial effectiveness of both formulations at two different doses (6mg and 125mg) against common pathogens: *staphylococcus aureus*, *pseudomonas aeruginosa*, *escherichia coli*, and *candida albicans*. Results indicated that GSZnO NP showed higher sensitivity to *staphylococcus aureus* followed by *pseudomonas aeruginosa* and *e.coli* at lower concentration compared to *yashada bhasma*, whereas *yashada bhasma* exhibited greater effectiveness against *candida albicans* at two different doses. These findings highlight the potential of *yashada bhasma* in treating fungal infections and the suitability of GSZnO NP for bacterial diseases. The study underscores the integration of traditional Ayurvedic medicine with modern nanotechnology to address infectious pathogens, suggesting avenues for further research and clinical applications in healthcare.

Keywords: *Yashada bhasma*, GSZnO NP, MIC, Agar disc diffusion, Anti-microbial

INTRODUCTION:

Ayurveda, deeply embedded in Indian culture for centuries, highly esteems zinc, known as *Yashada*, for its medicinal properties. At the heart of *Ayurvedic* traditions are *bhasmas*, classified as metal-based remedies, which undergo a precise conversion process called *bhasmikarana* [1]. This technique transforms metals and minerals into a form easily absorbed by the body, termed *bhasmas*. This elaborate procedure entails several refining stages (*sansakaras*), such as *shodhana* and *marana*, where minerals and metals undergo repeated cycles of burning and grinding with herbal extracts and specific ingredients. The resulting product, "*Bhasma*," is a finely powdered metal-based remedy, distinguished by nanoparticles of nano dimensions [2].

Bhasma, purportedly consisting of biologically produced nanoparticles, is often included in various *Ayurvedic* medicines alongside other components [3]. Metallic preparations have become essential in *Ayurvedic* therapeutics owing to their added benefits such as reduced dosages and rapid efficacy [4]. *Yashada* (Zinc) is a prominent metal utilized in various forms for the treatment of diverse ailments. Zinc, the second most abundant transition metal found in organisms after iron, is distinctive for its presence in all classes of enzymes. In humans, zinc serves a vital role in biological

function, and its insufficiency is linked to numerous diseases [5]. In *Ayurvedic* classics, it is recommended for various conditions like diabetes (*Prameha*), anemia (*Pandu*), respiratory issues (*KasaSwasa*), night sweating (*Nisha sweda*), heavy menstrual bleeding (*Rajasrava*), Parkinson's disease-like symptoms (*Kampavata*) and Chronic non-healing wounds (*Dushtavrana*) [6].

Contemporary science research highlights the multifaceted role of zinc in supporting diverse bodily functions. It aids in bolstering the immune system, maintaining skin health, regulating blood sugar levels, and sustaining cardiovascular well-being. With its anti-inflammatory and antimicrobial properties, zinc helps prevent skin issues and respiratory infections. Moreover, it facilitates proper brain function, supports eye health, and potentially mitigates certain cancer risks [7].

In recent years, infectious diseases have become increasingly prevalent worldwide, with the pandemic exacerbating this trend. In the realm of medicine, Nano medicines, especially Nano-drug delivery systems, are gaining traction as an emerging field. Despite the current popularity of nanoparticles, ancient texts have long discussed the use of *bhasma*, which operates in Nano form and is recognized for its rapid efficacy with minimal doses.

Green synthesis of nanoparticles refers to the environmentally friendly approach of synthesizing nanoparticles using natural sources, such as plant extracts, microorganisms, or other eco-friendly materials which act as a reducing agent in preparing nanoparticles [8]. The green synthesis technique for producing ZnO NP using aloe vera leaf extract closely resembles the process used to make classical *yashada bhasma* (*vanaspati marita yashada bhasma*) where *kumarai swarasa* used as *bhavana dravya*. Recent findings indicate that *yashada bhasma* contains zinc oxide nanoparticles [9].

The pharmaceutical advantages in terms of synthesizing ZnO NP using green synthesis approaches are relatively simpler methods compared to the traditional processing of *Yashada bhasma* (ZnO NP). But, clinical evidence for usage of GS ZnO NP is lacking. However, a thorough scientific understanding is essential to establish their safety and efficacy. *Yashada bhasma* is in practice and also has well established safety efficacy data and their synthesis methods are also confirming stable ZnO NP using instrumental analysis like XRD and SEM-EDAX [10, 11]. Previous research works done on *yashada bhasma* and green-synthesized zinc oxide nanoparticles (GS ZnO NP) have demonstrated antimicrobial properties [12, 13]. However, a comparative analysis of

their efficacy remains to be explored. Thus, this study seeks to compare their antimicrobial effects.

AIMS & OBJECTIVES:

The study aimed to compare the antimicrobial activity of *Vanaspati marita yashada bhasma* and GSZnO NP using aloe vera leaf extract.

MATERIALS & METHODS:

The Raw material Zinc (*yashada*) was procured from Shree Dhootpapeshwar Ltd., to prepare *vanaspati marita yashada bhasma*. The chemical reagents, zinc sulfate heptahydrate, and sodium hydroxide were purchased from KLE College of Pharmacy, Belgaum to prepare zinc oxide nanoparticles. The *Aloe-Vera* was procured from natural habitat. The preparation of *yashada bhasma* was carried out at PG Dept. of Rasashastra & Bhaishajya Kalpana, KAHER's Shri B. M. Kankanawadi Ayurveda Mahavidyalaya, Belagavi. The preparation of zinc oxide nanoparticles was carried out at the Department of Pharmaceutics, KLE College of Pharmacy, Belagavi. An anti-microbial study was carried out at the Microbiology Department, Maratha Mandal Dental College Belagavi.

Preparation of Yashada bhasma:

The unprocessed zinc (*Yashada*) underwent *samanya shodana* (General

purification) through the *dhalana* (poring the molten metal in a liquid media) method in five liquid mediums (*Tila taila* (Gingely oil), *Takra* (Butter milk), *Gomutra* (Cow, urine), *Kanji* (Sour gruel), *Kulattha kwatha* (Decoction of *Dolchius biflorus* Linn) for seven cycles each [14]. This was followed with *visesha shodhana* (Specific purification) with *churnodaka* (Lime water) for seven cycles, then subjected to *jarana* (Incineration) along with *aparmaga panchanga churna* (*Achyranthus apera* Linn), [15] and finally *marana* (calcined) with *Kumarai swarasa* (*Aloe-vera juice*) *bhavana* (trituration) until the desired characteristics of *bhasma siddhi* were achieved, accomplished at the fourth *puta* [16].

Preparation of Gs ZnO NP:

The prepared aqueous extract of aloe vera was combined with a solution of zinc sulfate heptahydrate, with a concentration of 10mm, and placed under a magnetic stirrer for 15 minutes. Following this, sodium hydroxide solution, with a concentration of 1M was gradually added drop by drop until white zinc oxide-suspended nanoparticles were obtained [13].

Anti-microbial study:

The evaluation of the Anti-microbial activity of *vanaspati marita yashada*

bhasma and GSZnO NP using *Aloe Vera* leaf extract was done with minimum inhibitory concentration and agar disc diffusion Method.

Minimum Inhibitory Concentration:

MIC is the smallest amount of an antibacterial substance, usually measured in mg/L (or µg/mL), that completely stops the growth of a specific organism under carefully controlled laboratory conditions [17].

Agar disc diffusion method:

The agar disc diffusion test, also referred to as the Kirby-Bauer method, is a commonly employed technique to assess how sensitive bacteria are to antibiotics. In this test, bacteria are evenly spread across the surface of an agar plate. Paper discs containing particular antibiotics are then placed onto the agar. As these antibiotics spread through the agar, they hinder the growth of susceptible bacteria, creating a clear area around the disc where growth is inhibited. The diameter of this area is measured and compared against standardized charts to determine the bacteria's susceptibility to the antibiotic [18].

Organisms:

The organisms used in the study are depicted in Table 1.

Table 1: Name of the organisms evaluated for Anti-microbial activity

Strains	Organisms Name
Bacterial strains	<i>e. coli, pseudomonas aeruginosa, staphylococcus aureus</i>
Fungal strain	<i>candida albicans</i>

Standard drugs:

The standard drugs used in the study are depicted in **Table 2**.

Table 2: Name of the standard drug used in this study

Organisms	Standard drug name
Bacterial	<i>Ciprofloxacin</i>
Fungal	<i>Fluconazole</i>

Stock solution preparation:

The stock solutions are prepared with two doses i.e., 6mg and 125 mg. The stock solutions are prepared in the form of suspension. The suspension of *yashada bhasma* and the suspension of GSZnO NP were prepared by the following method.

Preparation of dose at lower concentration (6mg/ml):

To get a 6mg/ml concentration, suspensions of *yashada bhasma* and GSZnO NP were prepared with the following measurements which is depicted in **Table 3**.

Table 3: Preparation of 6mg/ml concentration suspension of study drug

S. No.	Name of the Study Drug	Quantity taken	Distilled water	Tween 80	Concentration mg/ml
1.	<i>Yashada bhasma</i>	60mg	10ml	1gm	6mg/ml
2.	GS ZnO NP	60mg	10ml	1gm	6mg/ml

Preparation of dose at higher concentration (125mg/ml):

To get 125mg/ml concentration, suspensions of *yashada bhasma* and GSZnO

NP were prepared with the following measurements which is depicted in **Table 4**.

Table 4: Preparation of 125mg/ml concentration suspension of study drug

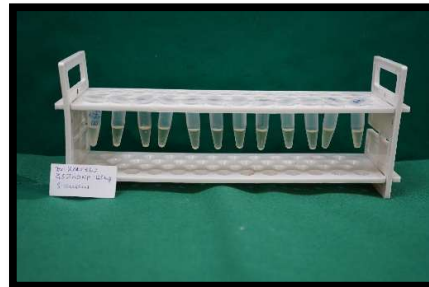
S. No.	Name of the Study Drug	Quantity taken	Distilled water	Tween 80	Concentration mg/ml
1.	<i>Yashada bhasma</i>	1250mg	10ml	1gm	125mg/ml
2.	GS ZnO NP	1250mg	10ml	1gm	125mg/ml

OBSERVATIONS & RESULTS:**Minimum Inhibitory Concentration:**

The inhibitory activity of both the study drugs is shown in **Figures 1, 2, 3, 4, 5**.



**Fig: 1 MIC of GS ZnO NP- 6mg
*Staphylococcus aureus***



**Fig: 2 MIC of GS ZnO NP- 125mg
*Staphylococcus aureus***



**Fig: 3 MIC of Yashada bhasma- 6mg
*Staphylococcus aureus***



**Fig: 4 MIC of Yashada bhasma-
125mg *Staphylococcus aureus***



**Fig: 5 MIC of Ciprofloxacin-
*Staphylococcus aureus***

The Results of MIC are depicted in **Table 5**.

Table 5: Results of study drugs along with standard drug in Minimum Inhibitory Concentration method

Sl. No.	Samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml	6.25 µl/ml	3.12 µl/ml	1.6 µl/ml	0.8 µl/ml		0.4 µl/ml	0.2 µl/ml
	<i>E. coli</i>											
	GS ZnO NP											
01	6mg	S	S	S	RT	RT	RT	RT	RT		RT	RT
02	125mg	S	S	S	S	RT	RT	RT	RT		RT	RT
	YB											
01	6mg	S	S	S	S	RT	RT	RT	RT		RT	RT
02	125mg	S	S	S	S	RT	RT	RT	RT		RT	RT
	Ciprofloxacin	S	S	S	S	S	S	S	S		S	RT
	<i>Pseudomonas</i>											
	GS ZnO NP											
01	6mg	S	S	S	RT	RT	RT	RT	RT		RT	RT
02	125mg	S	S	S	S	RT	RT	RT	RT		RT	RT
	YB											
01	6mg	S	S	S	RT	RT	RT	RT	RT		RT	RT
02	125mg	S	S	S	RT	RT	RT	RT	RT		RT	RT
	Ciprofloxacin	S	S	S	S	S	S	S	S		S	S
	<i>Staph</i>											
	GS ZnO NP											
01	6mg	S	S	S	S	S	S	S	RT		RT	RT
02	125mg	S	S	S	S	S	S	S	S		RT	RT
	YB											
01	6mg	S	S	S	S	S	RT	RT	RT		RT	RT
02	125mg	S	S	S	S	S	RT	RT	RT		RT	RT
	Ciprofloxacin	S	S	S	S	S	S	S	S		S	RT
	<i>Candida</i>											
	GS ZnO NP											
01	6mg	S	S	S	S	S	S	RT	RT		RT	RT
02	125mg	S	S	S	S	S	S	S	RT		RT	RT
	YB											
01	6mg	S	S	S	S	S	S	S	RT		RT	RT
02	125mg	S	S	S	S	S	S	S	S		RT	RT
	Fluconazole	S	S	S	S	S	S	S	S		S	S

S – Sensitive, RT-Resistant

Note: The lower dose of both the trial drug is 6mg and the higher dose is 125mg.

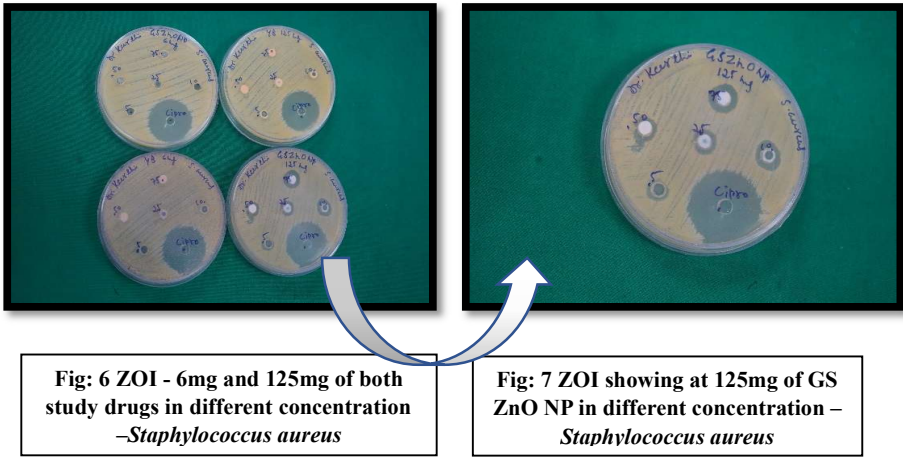
Observations:

Staphylococcus aureus exhibited notably higher activity when exposed to GS ZnO NP compared to *yashada bhasma* among bacterial strains. Conversely, *candida albicans*, a fungal strain, displayed significantly greater activity when treated

with *yashada bhasma* compared to GS ZnO NP.

Agar Disc Diffusion:

The zone of inhibition (ZOI) of *staphylococcus aureus* is shown in **Figures 6, 7**.



The Results of agar disc diffusion are depicted in **Table 6**.

Table 6: Results of study drugs along with standard drug in agar disc Diffusion method							
Sl. No.	Organisms	75%	50%	25%	10%	5%	Ciprofloxacin
	<i>E. coli</i>						
	GS ZnO NP						
01	6mg	RT	RT	RT	RT	RT	48mm
02	125mg	RT	RT	RT	RT	RT	40mm
	YB						
01	6mg	RT	RT	RT	RT	RT	35mm
02	125mg	RT	RT	RT	RT	RT	38mm
	<i>Pseudomonas aeruginosa</i>						
	GS ZnO NP						
01	6mg	RT	RT	RT	RT	RT	35mm
02	125mg	RT	RT	RT	RT	RT	32mm
	YB						
01	6mg	RT	RT	RT	RT	RT	38mm
02	125mg	RT	RT	RT	RT	RT	36mm
	<i>Staph</i>						
	GS ZnO NP						
01	6mg	RT	RT	RT	RT	RT	28mm
02	125mg	18mm	13mm	12mm	10mm	05mm	30mm
	YB						
01	6mg	RT	RT	RT	RT	RT	32mm
02	125mg	RT	RT	RT	RT	RT	35mm
	<i>Candida</i>						<i>Fluconazole</i>
	GS ZnO NP						
01	6mg	RT	RT	RT	RT	RT	35mm
02	125mg	RT	RT	RT	RT	RT	38mm
	YB						<i>Fluconazole</i>
01	6mg	RT	RT	RT	RT	RT	30mm
02	125mg	RT	RT	RT	RT	RT	32mm
RT-Resistant							

Observations:

Staphylococcus aureus exposed to 125mg/ml of GSZnO NP showed a subsequent increase in inhibition zones from 5mm to 18mm.

DISCUSSION:

Yashada bhasma is known for its potential to manage various health conditions including *rajayakshma* (tuberculosis), *dushtavrana* (chronic non-healing wounds) [6]. Despite, its established clinical use, there is a need to establish evidence for its antimicrobial activity, especially in the context of treating infective pathologies.

In contemporary science, Zinc oxide nanoparticles are also recognized for their antibacterial, anti-fungal, anti-diabetic, anti-oxidant, anti-inflammatory, anti-cancer activity and wound healing activities [19].

Zinc oxide, a common component in both *yashada bhasma* and Green synthesized zinc nanoparticles, has been identified through XRD analysis as a major component of the drug in both cases. Similarly, **Dr. Himanshu et al.**, confirmed zinc oxide nanoparticles by SEM-EDAX analysis of *yashada bhasma* and green synthesized zinc nanoparticles [9].

According to classical texts, three methods exist for preparing *yashada bhasma*: *paradamarita* (zinc metal processed and incinerated with mercury), *vanaspatimarita* (zinc metal processed and

incinerated with herbal drugs), and *arilohamarita* (zinc metal processed and incinerated with antagonistic drugs). In this study, the *vanaspatimarita* technique was utilized for the preparation of *yashada bhasma*. There are two different bhavana dravyas are explained in the context of *yashada marana* (calcination of zinc) like *nimbu swarasa* (lemonjuice) and *kumari swarasa* (aloe vera juice) [20, 21]. For the current study, *kumari swarasa* (aloe vera juice) was chosen as contemporary evidence was there for the GS ZnO NP. The use of aloe vera, in the process of synthesis acts as a reducing agent and also proven for its antioxidant and anti-microbial activity [13].

Further, *kumari swarasa* (aloe vera juice) in Ayurveda has been attributed to its *tikta* (bitter) and *madhura* (sweet) rasa, along with properties of *vishaghna* (detoxification), *jwarahara* (fever), *kasa*, *shwasa nashak* (alleviation of respiratory disorders), *kushthahara* (treatment of skin disorders), *krimihara* (treatment of infectious condition), and *tridosahara* (balancing of the three doshas). Scientifically, aloe vera has been proven for its antibacterial, anti-fungal, anti-diabetic, anti-oxidant, wound healing activity and anti-inflammatory action. The anthraquinones present in aloe vera act as potent anti-microbial agents. It also contains numerous trace elements, amino acids, and vitamins such as B1, B2, B3, B5, B6, and

B12, as well as essential minerals like calcium, manganese, zinc, phosphorus, and potassium [22]. These properties are added through bhavanasanskara (trituration) to the processing material zinc and the continuous grinding helped to reduce the particle size.

The anti-microbial activity using the MIC method confirms, that both the study drugs possess antimicrobial activity in varied concentrations for different microorganisms. In bacterial strains compared to the *yashada bhasma*, GSZnO NP has shown higher sensitivity in *staphylococcus aureus* at the lower dose of 6mg (6µl/ml concentration) and the higher dose of 125mg (0.8µl/ml concentration) whereas in *yashada bhasma* at both doses (6mg and 125mg) it has sensitivity from the concentration of 6.25µl/ml and comparatively at higher concentration in *pseudomonas aeruginosa* and *E. coli*.

In fungal strain compared to the GS ZnO NP, *yashada bhasma* has shown higher sensitivity in *candida albicans* at the lower dose of 6mg from the 1.6µl/ml concentration and at the higher dose of 125mg from the 0.8µl/ml concentration whereas in the GS ZnO NP has shown sensitivity at the lower dose from the 3.12µl/ml concentration and at the higher dose from the 1.6µl/ml concentration.

Similarly, an in vitro study of *yashada bhasma* utilizing the MIC method, exhibited remarkable antimicrobial efficacy against

both bacteria and fungi, even at the lowest tested concentrations. The minimum inhibition concentration (MIC) of *yashada bhasma* was found to be 3.12mg (3.12µl/ml) for *Bacillus cereus*, 0.78mg (0.8µl/ml) for *E. coli*, 1.56mg (1.6µl/ml) for *Staphylococcus aureus*, 0.78mg (0.8µl/ml) for *Pseudomonas aeruginosa*, and 0.78mg (0.8µl/ml) for *Candida albicans*. Compared to this study, our current research findings reveal a higher concentration sensitivity than those reported in their work. This variance could potentially be attributed to the utilization of the *paradamarita* technique in the earlier study. These comparative findings may be substantiated by the superiority of the *paradamarita* technique over the *vanaspati Marita* [12].

Even though the MIC has shown sensitivity at both doses for both the study drugs, it doesn't show any activity in the agar disc diffusion method except in *staphylococcus aureus* of higher (125mg). Previous research work by Asha Chaudary *et al.*, demonstrated GS ZnO nanoparticle has antibacterial effect against *e.coli* and antifungal effect against *aspergillus niger* in Agar disc diffusion method [13].

The no ZOI for both the selected drugs might be due to the dose selected for the study was comparatively less than the prescribed dose (*Yashada bhasma*) and the media selected (Span 20 and tween 80)

might not have released the drug from its suspension form.

The herbal components in both *yashada bhasma* and GS ZnO NP include phytochemicals like flavonoids, alkaloids, tannins, and saponins, known for their antimicrobial properties. These phytochemicals likely contribute to the antimicrobial activity observed in both drugs, as demonstrated by their Minimum Inhibitory Concentration (MIC) values [23].

CONCLUSION:

In conclusion, while both drugs demonstrate sensitivity across all strains, GSZnO NP exhibit higher sensitivity against bacterial strains compared to *yashada bhasma*. Conversely, *yashada bhasma* displays greater sensitivity against fungal strains than GSZnO NP. Considering *yashada bhasma* efficacy at lower doses (6mg), adhering to classical dosage references (62.5mg to 250mg) may optimize better patient outcomes. Consequently, *yashada bhasma* could prove more effective in treating fungal pathologies, whereas GSZnO NP may excel in addressing bacterial diseases.

Scope for further study:

Further study can be taken with increased dose along with minimum bactericidal concentration for both drugs.

Conflict of Interest: No conflict of interest

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