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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemistry and bioactivity of green synthesized nanoparticle from *Psidium guajava* against selected diabetic wound pathogens

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ABSTRACT
The present study is focused on Psidium guajava leaves extract impact on reduction of zinc oxide nanoparticle and to evaluate the antibacterial efficacy of ZnO against Gram positive pathogens collected from RMMCH. Leaves extraction done by cold extraction method and qualitatively analyzed. Green mediated zinc sulphate is reduced and characterized by UV, IR, SEM-EDAX. The antibacterial potential of ZnO NPs was examined by disc diffusion method. It was noted the leaves contain rich of phytochemical effectively reduced zinc oxide nanoparticle confirmed by Change in color of the reaction mixture from yellow to white indicated reduction confirmed by UV peaks at 348 nm corresponding to excitation of Zn nanoparticle. The synthesized nanoparticles were polydispersed, 20-100 nm and free from sulphate residues. FTIR reveals that plant metabolites contain alcohol, sulphate, nitrate, alkane functional groups also sustained in ZnO nanoparticles except sulphate. The green ZnO showed significant antibacterial activity against gram positive test pathogens comparatively higher than standard drug.
Keywords: gre*en nano, diabetic, zinc oxide, antibacterial, metal ions.*

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INTRODUCTION

Psidium guajava belongs to phylum Magnoliophyta, class Magnoliopsida and Myrtaceae family well known by a common name "Guava" an old history of medicinal value tree [1]. This plant grows widely in the tropic areas and has wide and clear green color leaves with clear prominent veins[2]. It contains containssaponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin and flavonoids along with Ascorbic acid and citric acid[3]. Guava leaves and bark extracts have high antibacterial activity that can inhibit the growth of *S. aureus, Bacillus*spand *Salmonella* bacteria[4]. In addition presence of quercetin derivatives on leaves contribute antidiarrheal activity similar to morphine like action and used to treat intestinal infection[5]. Guava is highly rich in antioxidants also show anti-nociceptive activity and thus helpful in decreasing the incidences of degenerative diseases[6]. Phytochemical of Guava leaves explored to develop various metal nanoparticles with multi-function. The green synthesis of metallic nanoparticles have been becoming the alternative routes of chemical and physical methods owing to lower costs, environmentally friendly and abundantly renewable resources[7]. Plant metabolites contain hydroxyl, carbonyl, and amine functional groups that interact with metal ions and reduce them as nanoparticle. Specially, flavonoids functional groups -OH group is mainly considered responsible for the reduction of metal ions into NPs[8]. Metallic nanoparticles have gained high attention because of their unique characteristics including surface plasmon resonance, thermo-optical properties and inherently antimicrobial capacity [9].ZnO is an inorganic compound which occurs rarely in nature. It is generally found in crystalline form. ZnO has been found to be potentially useful and efficient than other metals for biosynthesis of NPs for clinical purposes. Several studies have demonstrated the synthesis of ZnO NPs using different plant extracts with wide range of targeting pathogens [10-11]. The present study aimed to reduce zinc oxide nanoparticle using guava leaves against gram positive pathogen.

MATERIAL AND METHODS

Collection of medicinal plant

Leaves of guava (*Psidium guajava)* was collected and washed thoroughly in water to remove mud and dust particles. The leaves are shade dried and then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use.

Crude Extract Preparation

The aqueous extract was prepared by suspending 10g of powdered leaves in 200ml of distilled water. This mixture ultrasonicated for 300Hz for 3 cycle, and then allowed to stand for 24 hrs. The resulting extract was decanted and filtered through a Whatman filter paper. The filtrate was then concentrated with rotary evaporator at 45ºC.

Qualitative phytochemical analysis

Test for phenols and tannins

Crude extract was mixed with 2ml of 5% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids (Shinoda test)

One to five drops of concentrated hydrochloric acid (HCl) were added to little amount of ethanolic extract of the plant material. Immediate development of a red colour indicates the presence of flavonoids.

Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides

Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids

Test for quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for alkaloids

Two mL of extract was taken in a test tube and then 0.2 mL dilute HCl was included, followed by 1 mL of Meyer's reagent. A yellowish coloration indicates alkaloid's presence

Green synthesis of zinc oxide nanoparticles

10 mM zinc sulphate was prepared and 10 part (v/v) of zinc sulpahte was taken and mixed with two part of 0.1N KOH and then added with four part leaves extract under constant magnetic stirrer. The reaction temperature was maintained as 40° C. reduction of zinc oxide noted by colour change and confirmed by UV. Zinc added with KOH without extract maintained as negative control and zinc solution alone used as control.

Antibacterial activity of synthesized ZnONps

Test pathogen such as *Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus* were collected fromRMMCH. The antibacterial activity of the selected plant extract was determined by disc diffusion method proposed by Bauer et al. [12]. 25 µl (for 100 µl concentration) of the plant extract was loaded onto sterile empty discs of 6mm in diameter and allowed to dry under sterile condition. Mueller Hinton agar plates were prepared under sterile condition to use in the susceptibility test against the test bacteria. Using sterile swabsthe plates were inoculated with appropriate bacterial cultures. Bacterial pathogens using spread plate technique and incubated at 37ºC for24hrs.Antibiotic ciprofloxacin (20 µg) was used as positive control. zone of inhibition around each disk was measured and interpreted.

Recovery and characterization of ZnO nanoparticle

The precipitated reaction mixture was centrifuged at 3000 rpm and supernatant was taken for collection of nanoparticles. It was centrifuged at 10000 rpm for 20 min and the pellet is washed twice with ethanol (70%) and dried under oven for 1 h. the powder was subjected to SEM EDX. functional group was determined by FTIR spectrum.

RESULT AND DISCUSSION

The dried powdered *P. guajava* leaves extract showed presence of phytochemical on leaves extracts positive on tannins, alkaloids, saponins, cardiac glycoside, phenol tannin and flavonoids and absence of quinone, terpenoids and steroid ring (table 1). The data is maximum correlated with the work of Ekeleme Kenneth[13]. stated the presence of all phytochemical except the result of tannins, which is absent in this study due to ecological significance. Change of color from yellow to light brown precipitated was observed in extract added flask which is absent in controls. Changes in color of the reaction mixtures due to formation of ZnO NPs indicated that the leaf extracts have had reducing capacity and acted as capping agent[14].The UV Visible spectra of as synthesized Guava plant extract used ZnO NPs are shown in Fig1. As expected the absorption region of Guava 260 nm and Guava plant extract used ZnO NPs absorption region at 348nm. UV spectrum reveals formation wide band gaps at shorter wavelengths corresponding to oxide nanoparticle [15]. Figure 2 displays the FT-IR spectrum of pure Guava plant extract and plant extract used ZnO NPs. The Guava leaf extract showed nine different major peaks 3164, 1600(C=C), N-O stretching of 1541, 1507, alkane C-H bending at 1436 cm^{-1} addition to S=O stretching (1187 and 1313), 1105 and 1023 cm^{1} corresponding to C-N stretching. Where as ZnO showed only 6 different peaks recorded as 1561,1397,1348,1336, 1045, 1015 cm^{-1}(C=H fluoro compounds). It was noted that dissapearance of 3164 cm^{-1} a stron broad OH stretching. The characteristic peaks around 3651 $cm^{-1}$and 3416cm^{-1} are attributed to the O-H stretching mode of H-O-H group in the presence of water molecules.The surface morphologies of the guava plant used ZnO NPs**. Fig 3a-b** represents the SEM image of ZnO NPsobtained display spherical in shape which is uniformly distributed along the surface. The average grain size was found to be about 20 –100 nm, respectively. Fig .3c shows the EDX spectrum of ZnO NPs. SEM EDAX images further evidenced the NPs derived by*P. guajava* are entirely pure. Antibacterial effect of green mediated ZnO NPs showed good activity against *S aureus* and moderate against *Bacillus cereus* and *E.faecalis*. Both the *E. faecalis* and *S. aureus* showed highly sensitive than *Bacillus* sp. Table 2 shows the zones of inhibition of against pathogens compared with standard drugs and b ZnO NPS. Compare to standard antibiotic, ZnO NPs showed strong antibacterial effect with relative inhibitory zone of 89, 61and 80 % respectively among *S. aureus B.cereus and E.faecalis*. both Staphylococcus and Enterococcus were highly sensitive and *Bacillus* sp is moderately sensitive towards green ZnO. Similar report was observed on study of Biswas et al. [16]has reported that *P. guajava* has Gram-negativeand Gram-positive antibacterial characteristics.The zone of inhibition of Guava leeaves mediated ZnO NPs was significantly greater in comparison to standard cefixime antibiotic. These data suggest that the use green ZnO NPs found to be efficient to controll the Gram-positive pathogens like *S. aureus* infections.

Table 1. Qualitative phytochemical analysis of aqueous extract of Guava leaves

S.No	Phytochemical test	Interference	*Result*
1.	Saponins test	Stable foam- positive	Positive
2.	Phenols and tannins test	Black colour- positive	Positive
3.	Glycoside Salkowski's test (steroid ring)	Reddish brown- positive	Negative
	Keller Kiliani test (cardiac glycoside)	Brown ring- positive	Positive
4.	Flavanoid test- Shinoda test	Pink colour- positive	Positive
5.	Quinones test	Red colour- positive	Negative
6.	Terpenoids test	Red brown- positive	Negative
7.	Alkaloids test- Meyers test	Precipitation- positive	Positive

Table 2. Antibacterial activity of green zinc oxide nanoparticle

PATHOGENS	**ZONE OF INHIBITION (mm)**						
	25µg	50µg	75µg	100µg	Positive control	Negative control	RIZD %
S. aureus	-	10	12	21	19	4	89
B.cereus	-	8	11	16	18	5	61
E.faecalis	8	10	12	20	20	4	80

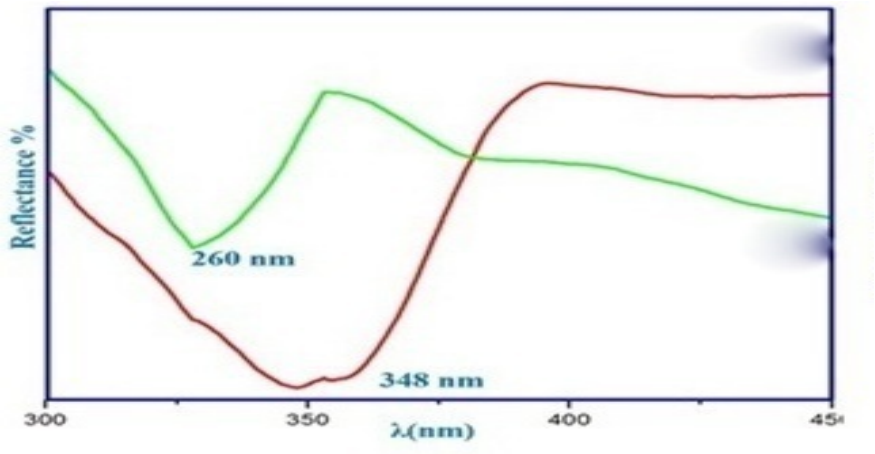


Fig.1. UV Spectroscopicalanalysis of zinc reduction

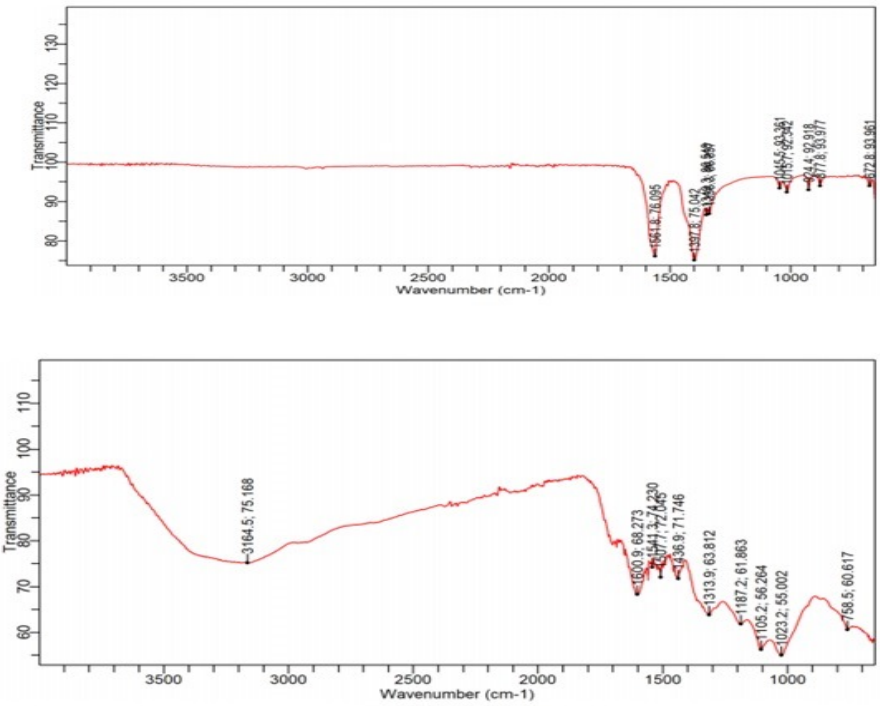
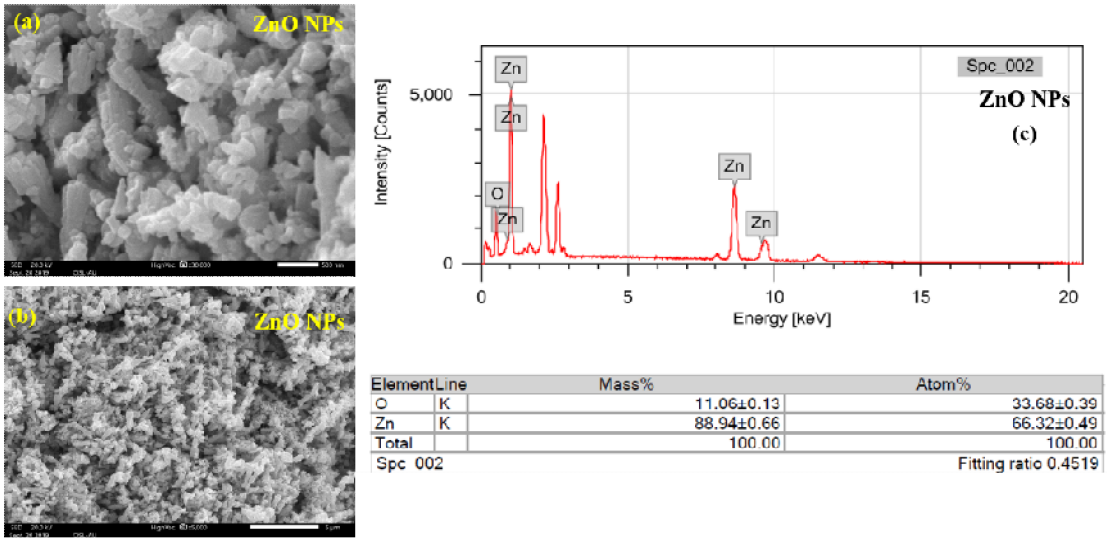


Figure 2 FTIR spectrum of reduced ZnO Nanoparticle and reducing agent



Element	Line	Mass%	Atom%
O	K	11.06±0.13	33.68±0.39
Zn	K	88.94±0.66	66.32±0.49
Total		100.00	100.00
Spc_002			Fitting ratio 0.4519

Figure 3. Shows the SEM and EDX spectrum of Gova plat extract used ZnO NPs

CONCLUSION

Leaf extracts of *P. guajava* showed presence of excellent reducing capping phytochemical in the formation of ZnO NPs with significant antibacterial effect against the gram positive wound infecting clinical pathogens.

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