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# Synthesis and characterization of silver nano particles from *Plectranthus ambionicus* extract and its antimicrobial activity against *Enterococcus faecalis and Candida albicans.*

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**Abstract**
Silver nano particles were prepared with *Plectranthus ambionicus* extract by boiling the leaves of *Plectranthus ambionicus* with silver nitrate. The stable silver nano particles were characterized by UV spectrum, which showed shift peak at 425nm. The antimicrobial activity of silver nano particles was investigated against *Enterococcus faecalis, Candida albicans* by disc diffusion method compared with standard drug. The result suggest that silver nano particles can be used as inhibitor against *Enterococcus faecalis*, *Candida albicans*, making them applicable in anti-microbial control system.

## INTRODUCTION

Incomplete disinfection of the complex root canal system may result in treatment failures and persistent apical periodontitis [1]. The bacterial penetration into the depths of 300 to 1500 µm in dentinal tubules [2]. The bacteria in these depths would remain inaccessible for conventional Irrigants, medicaments and sealers[3,4]. Enterococcus faecalis is a facultative gram positive bacterium, isolated mostly from the root-filled teeth with chronic apical periodontitis. Enterococcus Faecalis (E. Faecalis) has been found in 38% of the failed root canal-treated teeth .The ability to tolerate the rough environmental changes which is believed to be due to its high alkali tolerance [5] and tubular invasion ability of this cocci which protects it from intracanal endodontic medicaments, has made E. Faecalis a treatment-resistant microorganism. E. faecalis has the ability to invade the dentinal tubules and adhere to collagen and form a biofilm on dentin. E. faecalis can also survive high pH levels varying from 9 to 11 [6]. Nanodentistry implies the application of nanomaterials and dental nanorobots toward diagnosis and treatment, with the goal of improving comprehensive oral health. These nano-sized particles are different in properties such as active surface area, chemical and biological reactivity [7]. Nanomaterials offer unique physicochemical properties, such as ultra small sizes, large surface area/mass ratio, and increased chemical reactivity[8]. These advantages may be exploited to design highly specific materials and devices to interact with at the subcellular and molecular level of the human body in order to achieve maximal therapeutic efficacy with minimal side effects. In the field of endodontics, the development of nanomaterials is focused on steps that would improve antimicrobial efficacy, mechanical integrity of previously diseased dentin matrix, and tissue regeneration. Currently, newer technologies are being tested in endodontics, mainly toward overcoming the microbial challenge[9]. P.amboinicus belongs to Lamiaceae family and is known as country borage in English. P.amboinicus leaf extract may be used as a promising anti-microbial agent for the synthesis and characterization of silver nano particles.

## MATERIALS AND METHODS:

**Preparation of leaf extract:**

30g portion of Plectranthus ambionicus leaves were thoroughly washed and finely cut. Leaves were boiled in 100 ml sterile distilled water. The resulting extract was used for synthesis of nanoparticles.

**Synthesis of Silver nanoparticles**

25ml of ammonia was added to the 50ml of .01M silver nitrate solution followed by the addition of 50ml leaf extract. The concentration of silver nitrate was adjusted to .001M by making up the final volume to 500ml using distilled water. The observation of colour change after 24hr incubation in dark place indicates the formation of silver nanoparticles. These mixtures were centrifuged at 10,000rpm for 10minutes at room temperature. The pellet was collected and dried in hot plate. The dried powder was collected in Eppendorf tubes and stored in refrigerator and which was further characterized by spectrophotometer.

**Screening of antibacterial activity [Agar well diffusion technique]**

The nanoparticle powder was dissolved in distilled water in following concentrations 10mg/ml, 20mg/ml and 30mg/ml so that 100µl delivers 1000µg/ml, 2000µg/ml and 3000 µg/ml respectively. Broth culture of the bacterial strain and fungal strain compared to Mac Farland's standard 0.5 were prepared. Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates for bacterial and sabouraud dextrose agar for fungal, using sterile cotton swab and plates were dried for 15 minutes. Using a sterile well cutter wells measuring 4mm depth were made on the agar plates. 100 µl of different concentration of the extract were then filled in the wells. Calcium hydroxide was used as the positive control. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimeters. All the tests were done in triplicate to minimize the test error.

## RESULT:

Silver nanoparticles with Plectranthus ambionicus extract was prepared , which was evidenced by UV absorption spectra (Fig 1, Table 1)

**Fig 1: UV-spectrum of Plectranthus ambionicus silver nanoparticles**

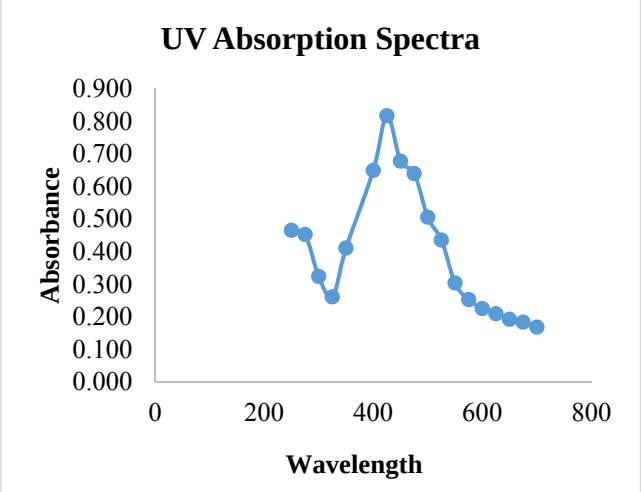


**Table 1:**

| Extract | Enterococcus faecalis | Candida albicans |
|---|---|---|
| 1000ug | 10.15±0.38 | 11±0.72 |
| 2000ug | 14.5±0.75 | 16.35±0.46 |
| 3000ug | 19.35±0.95 | 22.15±0.48 |
| Control group(calcium hydroxide) | 29±0.90 | 31±0.75 |

## DISCUSSION

In this in vitro study the antimicrobial efficacy of Nano silver particles with *Plectranthus ambionicus* extract and CH against E. faecalis. The antimicrobial activity of Nano silver particles with *Plectranthus ambionicus* extract was moderate compared to that of conventional CH. CH was rapidly killed the bacteria in the peterdish , only partial disinfection can be achieved in the surface wall of the root canal and CH was relatively ineffective .

The buffering capacity of dentin was proved by another study in which the effectiveness of CH paste against E. faecalis combined with different concentrations of dentin powder was evaluated [11]. E. faecalis resists high pH levels. It maintains a pH level by the buffering capacity of its cytoplasm. It also has a proton pump in which it provides additional homeostasis. However, studies have reported that this microorganism cannot resist pH levels over 11.5 [12] Therefore CH needs to penetrate dentinal tubules in enough concentrations to reach a pH of ≥11.5. The buffering capacity of dentin should also be considered in this regard [13]

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. Silver has more microbial efficacy and more effective in the presence of proteinaceous material.

## CONCLUSION

Silver nanoparticles incorporated extract *Plectrantus ambionicus* against *E.* Faecalis and Candida Albicans is less effective than calcium hydroxide. Therefore increasing concentration of the extract *Plectrantus ambionicus* in the nanoparticle can be assessed in future.

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