Original Research Article

Facile Green Synthesis of Iron Oxide Nanoparticles Using *Phoenix dactyliferaL.* Seed Extract and Their Antibacterial Applications

ABSTRACT

Aim: The synthesis methods of iron oxide nanoparticles (IONPs) have got great attention in recent years, due to their variety in physicochemical properties and applications. This study aimed to green synthesis the IONPs using aqueous extract of *Phoenix dactyliferaL*. seeds for its antibacterial application.

Methodology: IONPs were prepared in aqueous seed extract of *Phoenix dactyliferaL*. The physicochemical characterisations were performed with transmission electron microscopy (TEM) to study the shape of obtained nanoparticle, energy-dispersive spectroscopy (EDS) for elemental conformation of iron and oxygen, Dynamic light scattering (DLS) for particles size measurement, vibrating sample magnetometer (VSM) saturation magnetisation, Infrared spectroscopy (FTIR) for chemical confirmation of function groups and X-ray diffraction (XRD) for crystalline nature. The polyphenols contents were also determined; we suggest that the presence of phenolic compounds is the key element involved in formation and stabilisation of IONPs. Antibacterial activity was determined using disc diffusion assay and minimum inhibitory concentration of IONPs were determined by broth dilution assay.

Results: The biosynthesised IONPs were depicted to contain and crystalline magnetite spherical morphologies with average diameter 30 nm. Discs with all the concentrations of IONPs tested in this study (10 - 100 $ug.mL^{-1}$) showed antibacterial activity against bacterial strains tested (*Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). MIC values were found 30 $ug.mL^{-1}$, 50 $ug.mL^{-1}$ and 60 $ug.mL^{-1}$ for *S. epidermidis, K. pneumoniae* and *P. aeruginosa* respectively.

Conclusion: The biosynthesis of IONPs has provided, reliable, safe, simple and eco-friendly method. Hence, this study has focused on biological method of synthesis of IONPs. The IPNPs synthesized in this study can be used as therapeutic agents against bacteria.

Keywords: *Green nanoparticles, superparamagnetic iron oxide nanoparticles, antimicrobial efficacy, antibacterial activity,*

1. INTRODUCTION

Multifunctional metal oxides have emerged as promising materials in wide range of applications, such as biomedicines, contrast imaging, heavy metal removal, separation, cosmetics, diagnosis, catalysis and bioremediation [1]. In recent years, the interface of green chemistry and nanotechnology has provided an alternate approach of physicochemical methods of synthesis [2]. The biogenic methods are more eco-friendly, safer and more cost-effective for metal oxide nanoparticles productions. Plant extracts and microorganism are amongst the best promising approach for "green" synthesis, however aqueous plant extracts are preferred since their ease of availability and simplicity of use and scalability compared to biohazards and complex step of cell culture maintenances [3]. Aqueous plant extracts comprise a compelling array of antioxidants, such as polyphenols, tannins, alkaloids and flavonoids, amino acids, glycosides and nitrogenous bases that can reduce the metal ions in metal salt environment [4]. The reduction of metal ions leads to creation of nucleation points avoiding the further deposition. The incorporation of adjoining nucleation points subsequently leads to formation of nanoscale materials. Phytochemicals of plant extracts associate with such nanoscale materials providing a better stability. Such phytochemicals are hydrophilic, non-toxic and biodegradables and key elements for reduction and capping the resulting nanoscale materials. In addition, such nanoparticles have demonstrated lesser toxicity compared to nanoparticles synthesised using traditional physicochemical approaches. [4].

Beside the numerous studies on the synthesis of silver, zinc and gold nanoparticles using various plant extracts such as sorghum [5], Aloe vera [6], black tea [7], coffee [8] and fruit extracts [9], however efficient synthesis of IONPs using plant extracts is much difficult. This could be since reduced iron is rapidly oxidised in aqueous environment in comparison to its silver and gold counterparts causing a high degree of instability and aggregation. Moreover, IONPs have higher susceptibility to form agglomerates. This is because the large surface area to volume ratio of nanoscale IONPs increases the energy associated to surface area, a characteristic phenomenon may be aggravated with low surface charge. As a result, chemical and magnetic activities deteriorate and rapid clearance in biological system increases [10]. IONPs stability can be improved with electrostatic repulsion through surface coating or surface augmented ions [10]. IONPs have been preferred over other nanoparticles, due their ability to absorb and [11]. The heat or "hyperthermia" induces the cell apoptosis or sometime kills the cells. This promising feature of IONPs can be exploited in tumour therapy. Temperature > 38 °C to 45 °C has demonstrated the synergic efficacy of chemotherapy and radiotherapy [12]. IONPs generated heat produce over the exposure of magnetic field from the associated energy loss with Néel rotation and Brownian relaxation of magnetic moment of nanoparticles. Magnetic field induced heat generation can control the release of loaded payloads (therapeutics) at desired temperature range 38 °C – 45° C.

Previous studies demonstrated that IONPs synthesised with improved stability using tea, coffee and sorghum extracts. IONPs of different shapes and sizes were formed instantaneously in aqueous extract of tea. The resulting size and shapes of IONPs were dependent on the concentration of tea extracts, particularly these were hexagonal metallic

iron, magnetite, maghemite and amorphous iron [13]. Furthermore, 40 – 50 nm IONPs were produced in sorghum bran extract. These are well-reported to have higher amount of freely available polyphenols [5]. The biogenic IONPs were evaluated to be non-toxic in comparison with IONPs produced using traditional $NaBH_4$ and NH_4OH reduction methods [14]. Moreover, such biogenic IONPs were reported as functional through their model applications such as drug delivery and organic pollutant degradation [15]. Despite the IONPs synthesis in numerous plant extracts have been studied, this green synthesis approach needs an immediate improvement for optimisation of novel cheaper extracts to achieve stable, uniform size and shape. This would be conducive to obtain large scale synthesis of IONPs for biomedical, environmental remediation, hazardous waste treatment and hyperthermia applications [15].

In this study, we characterised the synthesised IONPs using iron chloride salt sources in *Phoenix dactylifera L*. seed extract. Furthermore, we evaluated the antimicrobial efficacy of biosynthesised IONPs in aqueous medium of *Phoenix dactylifera L.* seed extract.

2. MATERIALS AND METHODS

2.1 Plant extracts preparation and characterisation

*Phoenix dactylifera*L. (member of palm family Arecaceae plant) fruit seeds were used. Seed were ground into fine powder then water was added to 1 $g.L^{-1}$, which was mixed and heated at 80 °C for 30 min. The extract was filtered through muslin, the filtrate was centrifuged for 10 min at 1000 G. The supernatant was further filtered through 0.45 μm filters (Millipore) [16].

2.2 IONPs synthesis using date dectlefera seed extracts

IONPs were synthesised according to method [17] with small modifications. Iron source (mixture of $FeCl_2$ and $FeCl_3$ molar ratio 1:2) was used for this study. 0.1 M solution of mixture of $FeCl_2$ and $FeCl_3$ were also prepared with seed extracts. The solutions were mixed continuously at 80 °C for 2 h. The resulting suspension was vacuum filtered and black precipitates were collected. Afterwards, black precipitates were washed three times with distilled water and dried under vacuum. The synthesised IONPs were characterised using TEM, EDS, XRD, VSM, FTIR and DLS techniques.

TA LEO912 AB OMEGA transmission electron microscope (TEM) operating at 100 kV was used for image capture and selected area electron diffraction analysis (SAED) and EDS spectra data acquisition. The diluted (1:100) nanoparticles were droppedonto Formvar coated copper grids and left to dry at room temperature. EDS spectra were analysed simultaneously in TEM to achieve chemical composition of synthesised IONPs.

A Philips X'Pert Pro instrument was used for the X-ray diffraction analysis. The x-ray source consisted of Cu Kα radiation (λ= 1.54 A). Samples were scanning was performed at range of 20 - 70° within 2 Θ. The functional groups of IONPs were analyzed using Fourier transform infrared spectrophotometer (FTIR) (JASCO FT-IR 4700). Zetasizer Nano ZS (Malvern Instruments, UK) was used for the determination of size and surface charge of synthesised

IONPs using seed extract into 1 cm cell. The measurements were achieved using H-Ne laser (633 nm). Data were analysed using Dispersion Technology Software (DTS) version 5.10.

2.3 Antibacterial Activities

Antibacterial activity was determined using disc diffusion assay [18] against three bacterial strains (*Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*).Optical density of fresh cultures was standardised to 1×10^8 CFU.mL^{-1}. 100 µL of standardised culture was transferred on Mueller Hinton Agar (Oxoid) and uniform microbial lawn were prepared using sterilised glass spreader. Discs with 10 µL of IONPs (impregnated with 10 µg.mL^{-1}to 100 µg.mL^{-1}) were placed oneach bacterial lawn. A positive control was prepared using gentamycin (10 µg.mL^{-1}). Plates were incubated at 37°C for 24h. After incubation, the zone of inhibition was measured in mm. In addition to this, the minimum inhibitory concentration (MIC) of IONPs was also determined by broth dilution assay[19].

2.4 Total Phenolic Content

Total phenolic content was of seed extract was investigated using Folin-Ciocalteu's phenol reagent [18]. The absorbance was monitored at 725 nm (Jenway 6715, Spectrophotometer, UK). The total phenolic content was seeds extract was depicted in gallic acid equivalents (GAE) per gram.

3. RESULTS AND DISCUSSION

A rapid, simple, cost-effective, and green method for the biosynthesis of IONPs has been established successfully, using *Phoenix dactylifera* L seedextract for the first time. Bioinspired synthesis is attractive in health science applications compared to other physical and chemical methods. *Phoenix dactylifera* L seeds are already in use in folk nutrition and medicines. Phenolics, such as gallic acid, caffeic acid sinapic acid, synergic acid and tocopherols have been studied from *Phoenix dactylifera* L seeds [20].

3.1 Physicochemical Characterisation

TEM images were recorded to obtain the morphology of bioinspired IONPs. Recorded TEM images of IONPs depicted the spherical shape (Fig. 1 a). However, images also revealed that majority of nanoparticles were aggregated, this might be due to presence of hydroxyl groups from extract or thickening of extract. In addition, aggregation on IONPs (as Fe_3O_4) is not surprising due to ultrasmall size and magnetic properties [21]. DLS of synthesised IONPs showed average size of nanoparticles was 20 nm (±2.5 nm). The crystallite size from XRD pattern was 16.5 nm, which is in good agreement with TEM results.

FTIR spectroscopy revealed absorption band at 3445, 2925, 1675, 1235, 1065, 930, 845, and 705 cm^{-1} (Fig. 1 b), comparably, absorption bands of biosynthesized IONPs were observed at 3441, 2920, 1634, 1233, 1075, 958, 842, and 558 cm^{-1}. The peak at 3445 cm^{-1} in seed extract depicted vibration of O-H stretching. The band at 2925 cm^{-1} revealed the stretching vibration of CH_2 (C-H) (Yew). In IONPs, a new peak appeared that contributed to Fe-O. This metal oxide band corresponded to octahedral-metal stretching of Fe-O. The IONPs (Fe_3O_4) synthesis using seed extract are confirmed with peak at region between 400 to 600 cm^{-1} for metal oxide bonds [22].

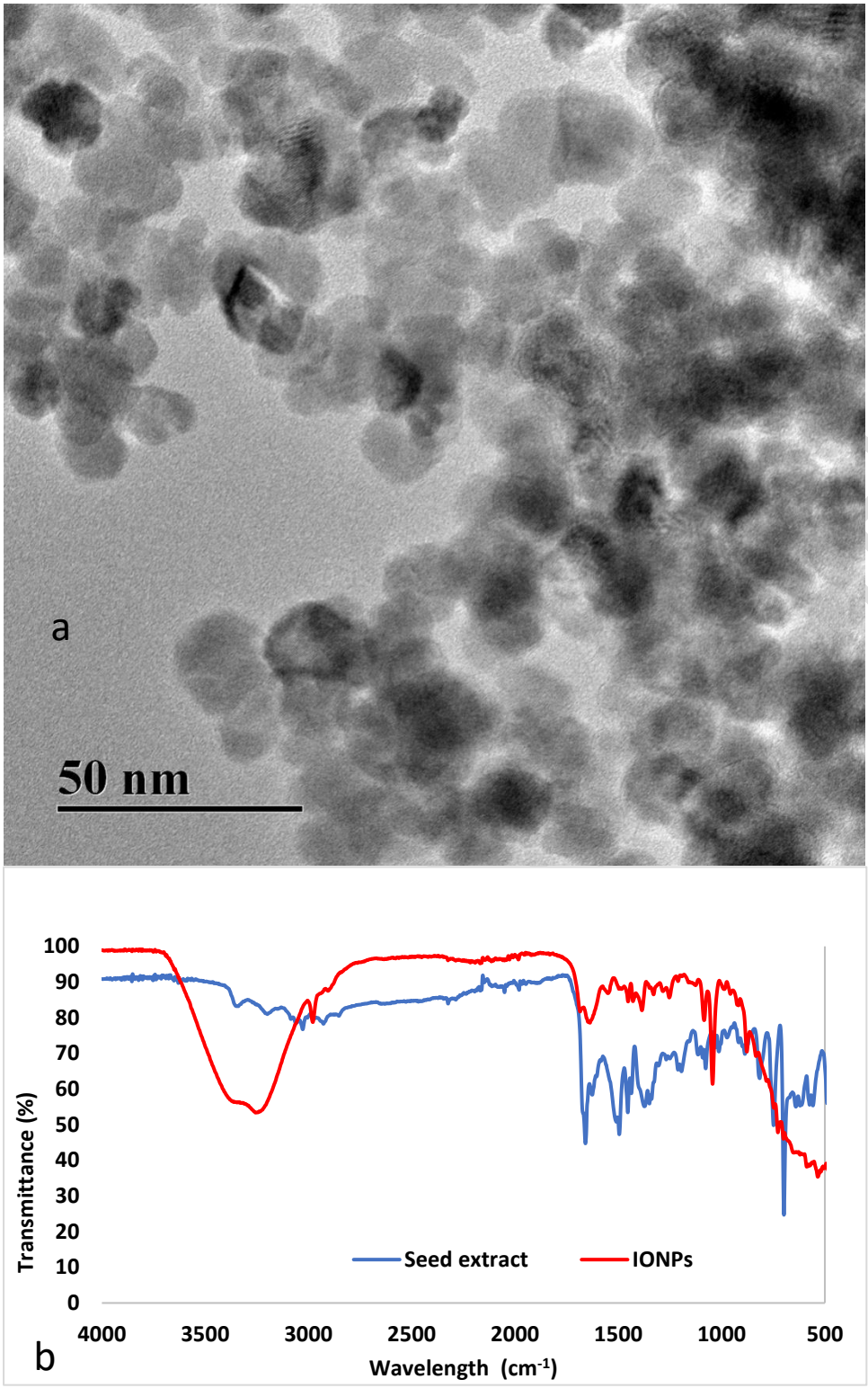

Fig. 1 (a)TEM image of biosynthesised IONPs (b) FTIR spectra of seed extract and bioinspired IONPs

XRD pattern of biosynthesised IONPs using seed extract is shown in Fig. 2 a. Pattern depicted 2 θ diffraction peaks at 0.4°, 35.8°, 43.5°, 54.1° and 57.4°, that contribute to

crystalline plane of (220), (311), (400), (511) and (440)for Fe_3O_4. The diffraction pattern is in good agreement with XRD standard of Fe_3O_4 in spinal phase structure [23].

Fig. b shows the saturation magnetisation measurements. The saturation magnetisation was 66.1 emu.g^{-1}. Coercivity (Hc) was negligible of hystress loop (5.6 kOe) and no remanence (1.3 emu.g^{-1}), which revealed the superparamagnetic behaviour of biosynthesised IONPs. Superparamagnetic property could be due to ultrasmall size on nanoparticle (20 nm) and could have only single domain.

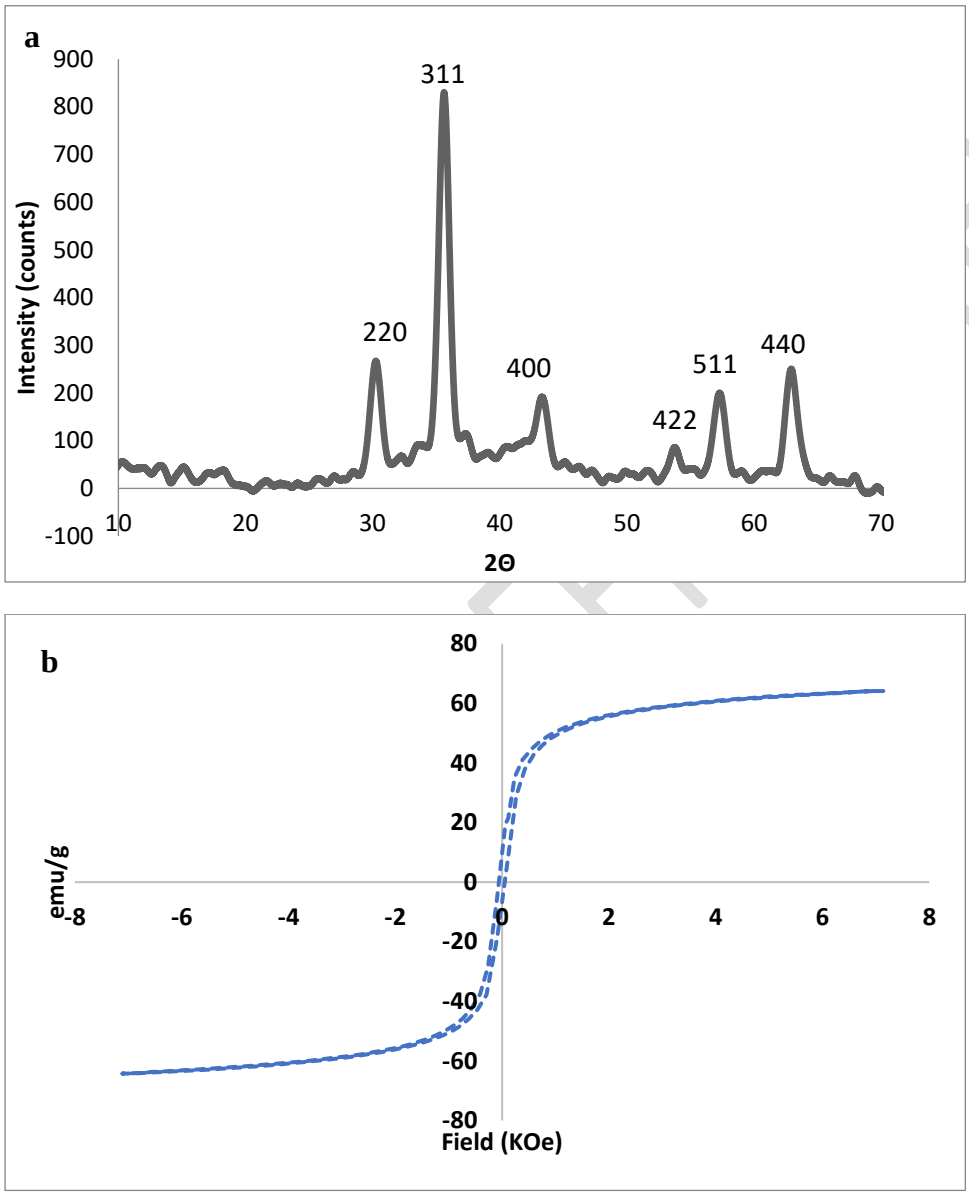

Fig.2. *(a) X-ray diffraction pattern of bioinspired IONPs (b) Magnetic hysteresis curves of IONPs*

3.2 Antibacterial Activities

Antibacterial activities were determined for green IONPs against three strains (*Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *P. aeruginosa*) using different concentration (10 - 100 ug.mL^{-1}) of discs and results are shown in Fig. 3. All strains were susceptible to bioinspired IONPs, where *S. epidermidis* was found least susceptible with MICs (30 ug.mL^{-1}), whereas for *K. pneumoniae* and *P. aeruginosa* MICs were 50 and 60 ug.mL^{-1}, respectively. It is pertinent to note that according to previous studies IONPs synthesised using chemical method showed higher MICs (50 mg). Similarly, IONPs synthesised with *Balanitesaegyptiaca* oil showed moderate antibacterial activity [24]. *Phoenix dactylifera L* seed have already been reported for antibacterial potential, now used for IONPs biosynthesis. This study revealed significant antibacterial activities against such pathogenic strains.

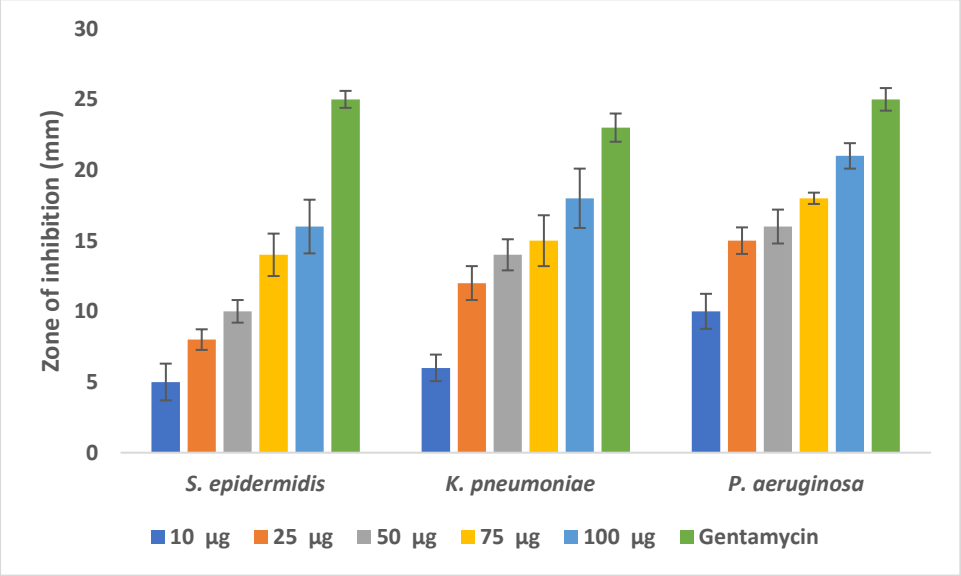

Fig. 3. Antibacterial activities of bioinspired IONPs

Numerous studies have explained the antimicrobial effect of IONPs synthesised using different approaches [25]. Most studies are focused on considering IONPs as generation of reactive oxygen species that lead to oxidative damage to cells as mode of action for their antimicrobial activity. Other researchers also considered non-oxidant factor *via* sorption of nanoparticles at membrane interface leading to perturbation of membrane bilayers [26]. Moreover, defects in surface of nanoparticles morphology could cause the membrane disruption or disorientation. This could increase the antimicrobial efficacy of nanoparticle up to ten-folds higher. We also explore the role of phenolics adhered from the aqueous *Phoenix dactyliferaL* seed extract have significant contribution to antibacterial potential.

4. CONCLUSION

Increasing attention for green chemistry and biological approaches has led to develop eco-friendly methods of synthesis of metal oxide nanoparticles. In contrast physicochemical process involves hazardous chemicals. A green chemistry-based approach for the biosynthesis of crystalline IONPs was demonstrated using eco-friendly, less expensive, rapid and simple method. The use of plants and waste products of agriculture as sustainable resources are advantageous to produce industry grade nanoparticles over other methods, such as use of microbes which are more costly due to their culture maintenance and purification cost. Detailed physicochemical characterisation and antimicrobial potency was performed. Our findings indicate the impressive potential of biosynthesised IONPs. IONPs have shown more antibacterial efficacy compared to those for chemically synthesised IONPs.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not Applicable.

ETHICAL APPROVAL

A written ethical approval from University has been obtained and preserved by the authors.

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