Formulation development of an herbal hand sanitizer containing *Moringa olifera* silver nanoparticles

Desenvolvimento da formulação de um higienizador de mãos à base de ervas contendo nanopartículas de prata *Moringa olifera*

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ABSTRACT

Background: The antimicrobial activity of *Moringa olifera* methanol leaf extract had been reported but with no effort to develop the extract into useful pharmaceutical products that is clinically relevant Objectives: This study aimed to develop an alcohol base herbal hand sanitizer containing synthesized Moringa silver nanoparticles as the active ingredient for use in personal hygiene and for combating the spread of outbreak of communicable diseases Methods: 10 mL of Moringa leaf extract (MLE), obtained by macerating the dried leaves with methanol was reacted with (40,30,20 and 10 mL) of 1mM silver nitrate solution to produced batches of Moringa silver nanoparticles (MSN). The morphology of MSN was obtained using a scanning electron microscope, while the particle sizes, polydispersity index and the zeta potential were obtained using a ZS-90 Zetasizer with dynamic and electrophoretic light scattering capabilities. MSN antimicrobial action was evaluated by agar diffusion method sequence to formulation of the herbal hand sanitizers, which were the evaluated for their effectiveness to reduce the microbial population. Results and Discussion: The color changes indicating the formation of MSN were within 10 minutes, all other MSN parameters significantly varied from batch to batch,($p \leq 0.05$) indicating the need of process optimization. The MSN were moderately dispersed, negatively charged and stable with PDI and ZP values ranging, 0.11-0.39 and 22- 33 mV respectively. FA and FB with yields above 50 % and mean particle sizes of about 30 and 38 nm were selected for production scale up and formulation studies. The percentage microbial death for FA1 and FB1 were 100 % showing an improvement above the standard of 99.9 % microbial death. Conclusion: Alcohol base herbal hand sanitizers were successful formulated with synthesized MSN which demonstrated an improvement above the industrial standard with ability to eradicate microbial load by 100 %. These novel herbal hand sanitizers could be readily deployed to combat the spread of communicable disease outbreak like the current covid-19 pandemic.

Keywords: antimicrobial activity, green synthesis. Hand sanitizing and *Moringa olifera*

RESUMO

Antecedentes: A atividade antimicrobiana do extrato de folha de metanol da Moringa olifera havia sido relatada, mas sem nenhum esforço para desenvolver o extrato em produtos farmacêuticos úteis que sejam clinicamente relevantes Objetivos: Este estudo visou desenvolver um anti-séptico de mãos à base de álcool contendo nanopartículas sintetizadas de Moringa prata como ingrediente ativo para uso em higiene pessoal e para combater a propagação de doenças transmissíveis Métodos: 10 mL de extrato de folha de Moringa (MLE), obtido pela maceração das folhas secas com metanol foi reagido com (40,30,20 e 10 mL) de solução de nitrato de prata 1mM para produzir lotes de nanopartículas de Moringa prata (MSN). A morfologia do MSN foi obtida utilizando um microscópio eletrônico de varredura, enquanto os tamanhos das partículas, o índice de polidispersidade e o potencial zeta foram obtidos utilizando um ZS-90 Zetasizer com capacidade de dispersão de luz dinâmica e eletroforética. A ação antimicrobiana do MSN foi avaliada pela seqüência do método de difusão do ágar para a formulação dos higienizadores de mãos à base de ervas, que foram os avaliados quanto à sua eficácia para reduzir a população microbiana. Resultados e Discussão: As mudanças de cor indicando a formação do MSN foram dentro de 10 minutos, todos os outros parâmetros do MSN variaram significativamente de lote para lote,($p \leq 0,05$) indicando a necessidade de otimização do processo. Os MSN foram moderadamente dispersos, carregados negativamente e estáveis com valores de PDI e ZP variando, 0,11-0,39 e 22- 33 mV respectivamente. FA e FB com rendimentos acima de 50% e tamanhos médios de partícula de cerca de 30 e 38 nm foram selecionados para estudos de escala de produção e formulação. A porcentagem de morte microbiana para FA1 e FB1 foi 100% mostrando uma melhora acima do padrão de 99,9% de morte microbiana. Conclusão: Os higienizadores de mãos à base de álcool foram formulados com sucesso com MSN sintetizado que demonstrou uma melhoria acima do padrão industrial com capacidade de erradicar a carga microbiana em 100%. Estes novos higienizadores de mãos à base de ervas poderiam ser prontamente utilizados para combater a propagação de doenças transmissíveis como a atual pandemia de covid-19.

Palavras-chave: atividade antimicrobiana, síntese verde. Higienizador de mãos e Moringa olifera

1 INTRODUCTION

Moringa oleifera plants belong to the family Moringaceae. It is an economic tree commonly found in tropical and some sub tropical countries. Moringa today is and economical tree of great usefulness (household and health benefits). The plant has several local names such as the drum stick, miracle tree, horseradish tree etc, [1, 2]. All the various parts of the plants are consider useful and all are reputed to have various traditional and health benefits including treatment and management asthma, diabetics, obesity hypertension etc. In northern Nigeria and some parts of Asia the boil leaves of Moringa are consumed for its nutrient values as it's reputed as excellent source vitamins and minerals [3, 4]. *Moringa oleifera* leaves are also used for the treatment of colds,

diabetes, as wound healing, as anti oxidant, analgesic, asthma, diarrhea, sore throat and as anti-inflammatory agents [5, 6, 7, 8]. Some of these uses are still under scientifically investigations but its antimicrobial, wound healing, anti –oxidant, analgesic agent are scientifically demonstrated and documented, [9, 10, 11, 12, 13].

Fig. 1 *Moringa olifera* leaves (www.hekmacenter.com)

Green synthesis of metallic nanoparticle had received considerable global attention due to its unlimited possibilities and applications as well as its cost effectiveness and its extremely eco friendly process which is devoid of environmental contamination with heavy metals as byproducts, [14,15]

Silver nanoparticle is of interest to our research group for its various possible therapeutic uses that we hope to explore clinically. Green synthesis protocol of Lateef *et al.*, 2016 was adopted in this study to synthesized Moringa silver nanoparticle through the reduction of the silver nitrate solution, [16]. Also evaluating silver nanoparticles for potential drug development is of interest because some researchers had reported that green synthesized nanoparticles potentiate the pharmacological activities of the plant extracts, meaning lower doses of the silver nanoparticles will be required for therapeutic activities [17, 18]. In order to harness these useful potentials this study aimed to synthesize and evaluate Moringa silver nanoparticle (MSN) in the formulation of herbal hand sanitizer. This is of great interest because since the outbreak of the current corona virus disease (COVID-19) the demand for the hand sanitizers had skyrocketed and the prices beyond the reach of the common man. Also the supply chained of some active ingredients heavily unsecure and some manufacturers are closing up due to shortage of raw materials [19]. There is therefore the need to explore the antimicrobial potential of some Nigeria medicinal plant as active medicinal agent in the production of herbal hand

sanitizers to meet the rising global demand for hand sanitizers, since hand sanitizing remained one of the most effective methods of preventive and controlling the transmission of communicable diseases [20, 21].

2 MATERIAL AND METHODS

Moringa olifera leaves were obtained from the botanical garden of the department of pharmacognosy and ethnopharmacy, faculty of pharmaceutical sciences Usmanu Danfodiyo University Sokoto. All other reagents are analytical grade.

Extraction, phytochemical and microbial screening of Moringa olifera leaf

Fresh *Moringa olifera* leaves (fig.1) were collected early in morning (before sun rise) from the botanical garden of the department of pharmacognosy and ethnopharmacy, faculty of pharmaceutical sciences Usmanu Danfodiyo University Sokoto and were open air dried at temperature not above 25^{O}C.The dried leaves were thereafter size reduced. From which 25g was measured and transfer into a 250-ml glass beaker along with 200 ml of methanol for maceration for about 24 hours. The obtained methanol extract (MLE) was filtrated using filter paper (Whatman No. 1 Maidstone, UK). The filtrate was thereafter subjected to centrifugation at 1,200 rpm for 5 min for the removal of heavy biomaterials. The obtained MLE was divided into three portions. A portion was subjected to standard phytochemical screening to determine its bio-composition, while the second portion was used for antimicrobial activity screening using the agar diffusion technique, however the last portion was stored at room temperature for green synthesis of Moringa silver nanoparticles (MSN) [22, 23]

Biosynthesis of Moringa silver nanoparticles (MSN)

The green synthesis was conducted in batches and at ambient laboratory temperature of about 33 ± 2 ^{O}C (table 1). The required volume of MLE and 1 mM aqueous solution of silver nitrate (AgNO3) solutions were held under continuous magnetic stirring at 500 rpm until colour the colour of the reactant solution changed signifying formation of MSN [24]. The surface plasmon resonance of MSN was determined using a UV-visible spectrophotometer operated between 300 -700 nm. The synthesized MSN were recovered by ultracentrifugation operated at 13,400 rpm for 30 minutes at 4°C and were thereafter freeze dried (FD-10-TP Labfreeze Instrument, Hunan China), [25]

Table 1: Batch formulary for MSP

Materials	FA	FB	FC	FD
MLE (mL)	10	10	10	10
AgNO3 (1mM) (mL)	40	30	20	10

Morphology of MSN

Samples of the produced MSN were spread on metal stub and were gold coated using ion sputtering device and thereafter vacuum dried. The external morphology of the gold coated MSN were obtained using a scanned electron microscope (SEM), (JEOL JSM 5200 Tokyo Japan), [26]

Particle size, polydispersity index (PDI), and Zeta potential

A ZS-90 Zetasizer with dynamic and electrophoretic light scattering capabilities (Malvern Instruments, Worcestershire, UK) was employed for the determinations of the mean particle sizes, PDI and the particle surface charges (Zeta potential) for all the batches of MSN produced. The determinations were done in triplicate at detection angle of 90 ^{O} and room temperature of about 25 ^{O}C [27, 28, 29].

Antimicrobial evaluations of synthesized MSN

Four clinical isolates (Escherichia *coli, Klebsiella granulomatis, Pseudomonas aeruginosa, and Staphylococcus aureus*) were obtained from department of pharmaceutics and pharmaceutical microbiology of this university. The test organisms were grown separately in peptone water for overnight and thereafter used in seeding of the sterile Mueller–Hinton Agar Plates using sterile cotton swap sticks. The seeded plates were labeled according to the organism contained. Wells were created aseptically within the plates using a sterile cork borer (7 mm diameter), and thereafter irrigated with 100 µL of graded concentrations of test solutions (30 and 60 µg /mL). The plates were labeled accordingly and incubated for 24 h at 37 ^{O}C. The procedure was equal repeated for co-amoxical® as positive control. At the end of incubation, plates were examined for the zones of inhibition, which were measured and recorded [30, 31].

Formulation of herbal hand sanitizer (HHS)

Alcohol base and non alcohol base hand sanitizers containing FA/FB were prepared in batches according to table 2. Standard formulation procedures were observed

for each unit operations such as weighing, trituration, dissolution, mixing and labeling. The control and WHO were devoid of MSN table 2.

Table 2: Batch Formulation of HHS

Materials	**FA_1**	**FA_2**	**FB_1**	**FB_2**	**WHO**	**Control**
MSN (mg)	10 mg	10 mg	10 mg	10 mg	Nil	Nil
Glycerol (mL)	1	1	1	1	1. 6	1
Fragrance (mL)	0.5	0.5	0.5	0.5	PRN	0.5
H_2O (mL)	15	100	15	100	Nil	15
Ethanol (mL)	83.5	Nil	83.5	Nil	93.7	83.5
3% H_2O_2 (mL)	Nil	Nil	Nil	Nil	4.7	Nil

Comparative microbiological evaluations of HHS and WHO formulations

Sixty healthy volunteers with no skin sores were employed for the microbial reduction evaluation test. The participants were grouped into six groups, each group were use to evaluate each of the hand sanitizer formulations, (table2). Sterile cotton swap sticks (A) were used to swap the two hands before the application. New sticks (B) were used to re-swap the hands after application and rubbing of the sanitizer. The microbial cell viability assay technique was carried out using the BacTiter-Glo™ test kit. The kit provides a rapid method for estimating the number of viable microbial cells in the culture based on quantification of the ATP present. The homogeneous assay involves adding a single reagent (BacTiter-Glo™ Reagent) directly to bacterial cells cultured in medium and measuring luminescence. Both A and B swap sticks were culture in different Mueller Hinton II (MH II) Broth at 37°C overnight. The overnight culture was diluted $1:10^6$ in 50ml of fresh MH II Broth and incubated at 37°C with shaking at 250rpm for 12 hrs. The BacTiter-Glo™ Assay was performed according manufacturer protocol. The Luminescence was recorded on a GloMax® 96 Microplate Luminometer, [32]. The percentage viable cells for the various batches of hand sanitizer were determined using the expression below;

$$Q = \frac{\text{Number of Viable cells from B}}{\text{Number of Viable cells from A}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq1}$$

Where Q is the % viable cells

3 RESULTS AND DISCUSSION

Extraction and phytochemical screening of MLE

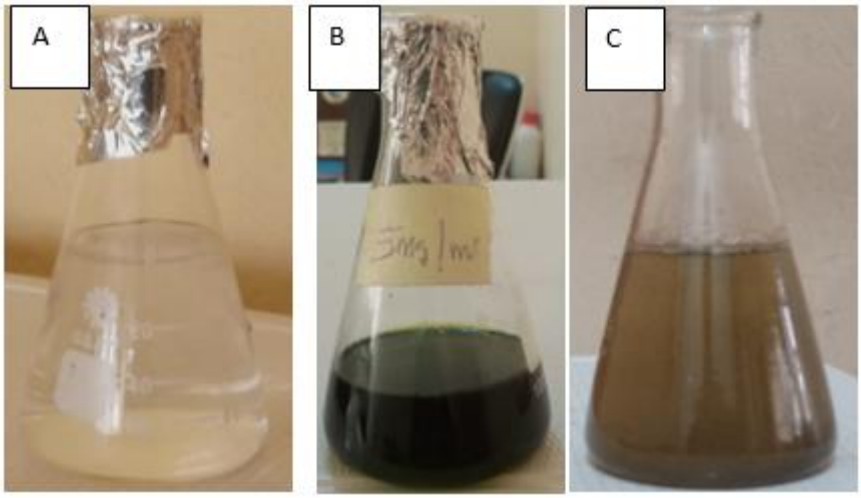
The methanol extract of Moringa olifera leaves yield was about 38% w/w. The phytochemical screening revealed the presence of secondary plant metabolites such as

alkaloids, tannins, flavonoids, terpenoids, saponins, glycoside, anthraquinones, and compounds reducing sugars. MLE also shows antimicrobial activities against all test organisms as earlier reported by several authors, [33, 34]. This current investigation aimed to translate basic scientific information to useful novel pharmaceutical product.

MSN biosynthesis

The use of Moringa leaf extract (MLE) as a reducing agent to synthesized MSN represent a simple and one step pathway which can be adopted for most metallic nanoparticles as the process is devoid of impurities, toxic residue and its economically viable [31]. The biosynthesis of MSN was rapid and completed within five to Ten minute of adding the aqueous silver nitrate accomplish with visible colour change fig.2 & 3.

Fig 2 (a-c): (a) 1mmole $AgNO_3$ solution, (b) MLE (c) MSN.



The yields of MSN for FA and FB were 58 and 52 % respectively. The observed differences are reactants proportion dependent and this is significant, ($p \leq 0.05$). However, the yields of these two batches were high enough for mass production of MSN using the biosynthetic technique and at specified proportions. The yields of FC and FD were low (about 29 and 25 % respectively); these batches were considered not economically viable and were not selected for formulation studies, [35]. Vis Uv spectroscopy was use to monitored the biosynthesis of MSN and the maximum absorbance peak was observed at same wavelength (418 nm), figure 4. The maximum absorbance peaks for green synthesis of silver nanoparticles reported in the literature range from 391 to 440 nm, [36, 37]. These differences may be due to diverse phytochemical compositions of the plant extracts used for biosynthesis.

Fig. 3: Schematic green synthesis of MSN.

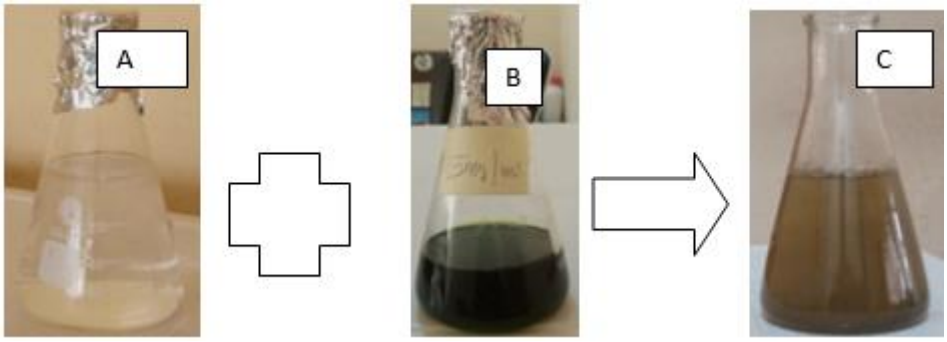


Figure 4; UV-Vis absorbance study

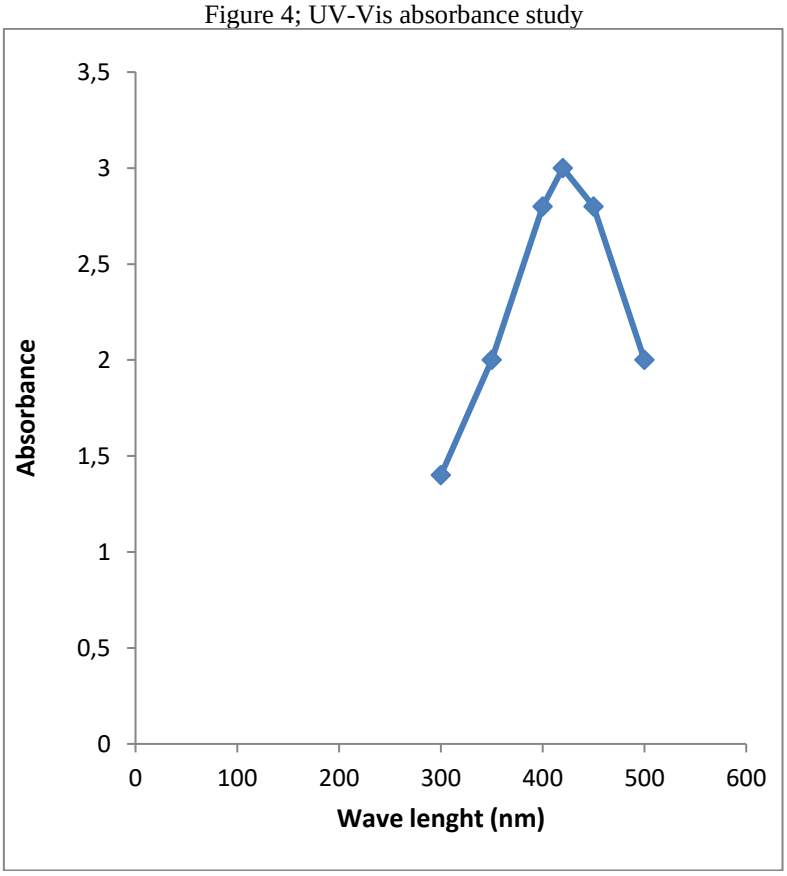


Table 3: Some parameters of biosynthetic MSN

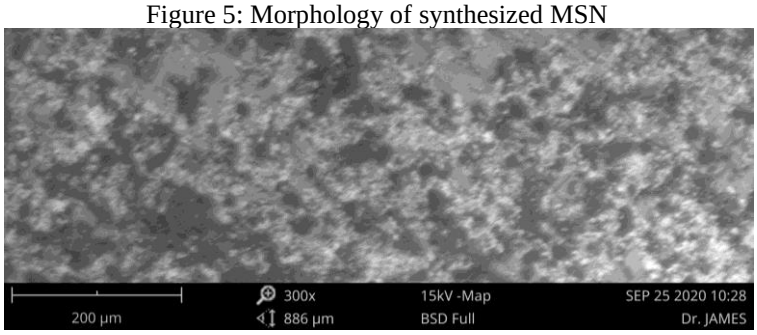
Matches	Yield (%)	AV. Diameter (nm)	PDI	ZP (mV)
FA	58 ± 0.04	30 ± 0.11	0.39 ± 0.02	-22 ± 0.11
FB	52 ± 0.14	38 ± 0.14	0.37 ± 0.04	-24 ± 0.14
FC	29 ± 0.08	43 ± 0.06	0.18 ± 0.16	-30 ± 0.03
FD	25 ± 0.06	46 ± 0.04	0.11 ± 0.16	-33 ± 0.04

Morphology, particle size, PDI and zeta potential of MSN

Reported mean particle sizes of silver nanoparticles ranged between 3and 50 nm, depending on the biosynthetic conditions and the plant extract utilized, [37]. SEM of MSN revealed images of different dimensions which were mainly spherical in nature with mean diameter ranging from 30 to 46 nm, fig 5. The observed differences in the mean

particle sizes were attributed to concentration of MLE used, since that was the only differential factor during the synthesis. An inverse proportionality was observed between the concentration of MLE and the average particle sizes of MSN in which case FC and FD (low MLE concentration) had higher particle sizes as compared to FA and FB. The mean particle sizes represent one of key determinant of therapeutic activities of nanoparticles [38]. The polydispersity index PDI and the zeta potential (ZP) of colloids are vital parameters to formulation scientists. While PDI is a measure of the size distribution of the nanoparticle, ZP measures the surface charges of the nanoparticle in solution. These two parameters go a long way in determining the stability of colloidal system [39, 40]. The PDI values for MSN ranged from 0.11 to 0.39 showing a moderately dispersed nanoparticulate system. PDI values above 0.4 are considered high poly dispersed, while values less than 0.1 are considered to be highly monodisperse system. In the same manner, ZP values between ± 0 and ±10 mVolt are considered unstable system that may lead to drug therapy failure. Values between ± 10 and ± 20 are classified stable system. While the colloidal formulation is classified as highly stable if the ZP value is between ± 20 to ± 40. These classifications are industrial standards and serves as essential guilds in pharmaceutical product formulation and development, [41, 42]. The ZP values of MSN ranged - 22 to – 34 indicating that green synthesized MSN are highly stable.

Figure 5: Morphology of synthesized MSN



Antimicrobial activity of MSN

FA, FB and the control (Co-amoxical®) inhibited the growth of the test organisms in dose dependent manner, table 4. The zone of inhibition (ZI) ranged, 12.8 -25.8mm. The ZI of FA and FB were significantly higher ($p \leq 0.05$) compared to the control. This may be due to nano sizes of FA and FB which may confers higher microbial cell wall penetration, nucleus destruction and death of the test organisms. Several authors had equally reported dose dependent antimicrobial activities of biosynthesized silver

nanoparticles, [43, 44]. Figure 6 is a selected plate showing the zone of inhibition of the synthesized MSN.

Figure 6: Selected plate showing ZI of synthesized MSN

Table 4: Zones of inhibition of FA, FB and control

Organism	Dose µg/mL	FA (mm)	FB (mm)	Control (mm)
EC	30.0	13.3	12.7	12.9
	60.0	19.8	18.3	16.8
PA	30.0	19.9	18.4	16.8
	60.0	25.8	24.9	20.1
SA	30.0	18.0	17.8	16.8
	60.0	22.1	19.3	17.9
KG	30.0	16.8	16.6	16.0
	60.0	20.9	19.6	18.4

EC, PA, SA and KG are *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella granulomatis*

Comparative antimicrobial actions of HHS and WHO hand sanitizer formulation

The world health organization had recommended an alcohol base hand sanitizer formulation containing hydrogen peroxide as the antimicrobial agent to fight the covid-2019 pandemic (WHO 2020). Hand sanitizer are use to reduced the microbial population by to 99.9 % within shortest possible period usually 30 seconds, [45, 46, 47]. FA1 and FB1are both alcohol base MSN formulations they dried up quickly and had zero number of viable cells.

Table 5: Percentage viable cells

Batch	% Viable cells
FA1	0.00
FA2	0.01
FB1	0.00
FB2	0.02
WHO	0.00
CONTROL	0.04

FA2 and FB2 are non alcohol formulations which had 99.9 and 99.98 % respectively. The failure of the FB2 to achieved satisfactory antimicrobial action may be due to the mean particle size of its MSN which are comparatively higher as compared to FA formulations. The mean particle size was reported to quantitatively and significantly affect the therapeutic responses of doxorubicin nanoparticle formulations, [48]. There is therefore the need to carefully optimize the biosynthetic conditions. The result clearly shows that novel alcohol base herbal hand sanitizer formulations evaluated in this study met CDC and FDA specification (of reducing microbial population by 99.99%) and serves as a credible alternative. Also their antimicrobial actions are comparable with the WHO recommended formulation and may be deployed in combating the current and future disease outbreak of this nature.

4 CONCLUSION

In this study we were able to successfully formulate three novel herbal hand sanitizers containing MSN. The method of preparation is simple and can be easily learn even by non-healthcare professionals. The antimicrobial activities of the three successful formulations were comparable with that of WHO recommended alcohol base formulation. These novel herbal formulations may be readily prepared and deployed in the current battle against the current and future infectious disease outbreak.

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