Science World Journal Vol. 18(No 1) 2023
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

FORMULATION AND EVALUATION OF IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF HERBAL HYDROGEL LOADED WITH *MORINGA OLEIFERA* LEAF EXTRACT

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ABSTRACT
Herbal preparations, usually containing highly concentrated crude drugs of plants materials have been used traditionally to treat various disorders in developing countries including Nigeria. In this work, herbal preparation in form of hydrogels containing *Moringa oleifera* leaf extract were formulated and evaluated for phytochemicals, antioxidant and antimicrobial activities using standard procedures. The result obtained shows the total phenolic and flavonoid contents of the M. oleifera leaf extract to be 288.59±34.18 mg GAE/g and 242.19±32.40 mg GAE/g respectively. The result of the antioxidant activities revealed that HG2 (10%) hydrogel possesses higher scavenging activities compared to HG1 (5%) hydrogel at different concentrations. The antimicrobial activity of the hydrogels was found to be higher for GH1 (5%) in most bacteria across various concentrations while *Candida albican* showed higher sensitivity to HG2 (10%). Hydrogels are excellent drug carriers that ensure controlled release of active ingredients. An uncontrolled release of active component of herbal preparations and lack of proper dosage may contribute to adverse side effects recorded for traditional herbal preparations. Development of carrier systems that control the release of active ingredients of herbal drugs is strongly recommended in order to minimize the negative side effect of these preparations.

Keywords: Hydrogel, Moringa oleifera, Antioxidant, Antimicrobial.

INTRODUCTION

Medicinal plants remain a major source of medication in most developing countries. The practice of traditional or herbal medicine has become part of the lifestyles of the majority of people in those countries with medicinal plants serving as a major source of healthcare. It is estimated that between 50,000 and 70,000 plant species are employed globally in both traditional and modern therapeutic applications (Hayta *et al.*, 2014). Various parts of these plants; leaves, stem bark, roots, fruits, berries, and flowers, were found to be active in the treatment of different ailments using a variety of traditional methods. In Africa, more than 80% of the population relies on these plants for their primary healthcare (WHO, 2002).

The therapeutic properties of medicinal plants are related to the amount and the type of phytochemicals or secondary metabolites in the plant. The secondary metabolites are made of a wide range of bioactive substances, such as phenols, flavonoids, quinones, coumarins, phenolic acids, tannins, terpenes, and alkaloids which are responsible for the therapeutic properties of medicinal plants (Michalak *et al.*, 2021). These phytochemicals work synergistically to produce a combined effect that surpasses the total activity of the individual constituents, which tends to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body (Mahomoodally, 2013).
Moringa oleifera is a common and widely used plant in Nigeria. It is used both for culinary and medicinal purposes among all the ethno-cultural groups in the country. The Moringa leaves are rich sources of essential nutrients needed by the body such as proteins and vitamins, hence the plant can be used as a tool for combating malnutrition in populations where the deficiency of such nutrients is prevalent (Kou *et al.*, 2018).

Moringa tree is exceptionally rich in phytochemicals and this can be attributed to the diverse biological activities and disease preventive potential of the plant, consequently, there is a need to harness and utilize the chemical diversity of Moringa phytochemicals, which has significant potential for combating illnesses and improving health (Ma *et al.*, 2020). A number of medicinal properties of different parts of *M. oleifera* has been reported, from traditional or complementary medicine to scientific pharmacological studies, the plant was proved to possess a strong medicinal properties against various form of ailment ranging from diabetes, obesity, esophageal cancer and Atopic dermatitis (Lin *et al.*, 2018) and other forms of biological activities such as antioxidant, anti-inflammatory, hepatoprotective and lung-protective of extracts from various part of the plant *(*Lin *et al.*, 2018). Even more than how widely they are recognized and utilized, the methods by which herbal drugs are delivered can significantly affect the efficacy of such drugs. The therapeutic benefits of herbal medicines is determined by the concentration of their active ingredients; nevertheless, this concentration needs to be within a safe range because any levels above or below can be toxic or have no therapeutic effect (Singh *et al.*, 2021). In recent decades, researchers in biomedical fields focus in the development of novel drug delivery systems (NDDS) to optimize efficiency of herbal drugs and to control the release of active ingredients.

Hydrogels are hydrophilic, three-dimensional networks of cross-linked biocompatible polymers that can absorb a huge amount of water and undergo swelling and shrinking to provide controlled drug release (Narayanaswamy & Torchilin, 2019). Hydrogels loaded with herbal extracts were developed and studied for their possible application as materials for wound dressing and in the management of some skin diseases by many researchers (Lai & Rogach, 2017). The aim of this work was to formulate and evaluate herbal hydrogel loaded with *Moringa oleifera* leaf extract for its possible application in the control of skin diseases.

MATERIALS AND METHODS

Sample Collections

A sample of *Moringa oleifera* leaves was collected from a garden in Lokoja metropolis, Lokoja, Kogi State, Nigeria. The sample was taken to Herbarium Unit of department of Botany, Federal University Lokoja and it was identified and authenticated by Mr. Akanni T. Gbenga with voucher number 0208.

Sample Preparation

The sample was then dried under shade for two weeks, pulverized into fine powder using laboratory mortar and pestle. The powdered sample was kept in an air tight container at room temperature until further needed for extraction.

Extraction

Five hundred grams (500g) of powdered sample was cold macerated with 2000mL of 75% methanol for one week with periodic shaking. The sample was then filtered using No. 1 Whatman's filter paper, the filtrate was dried and concentrated to obtain hydro-alcohol crude extract. The crude extract was kept in refrigerator until needed for hydrogel formulation.

Formulation of *Moringa oleifera* Loaded Hydrogel

Preparation of Gel Base

To formulate *Moringa oleifera* based herbal hydrogel, procedure described by Ali *et al.* (2021) was adopted. One gram (1g) of Carbopol 940 was dissolved in 50 mL distilled water at 40–50^{o}C followed by 0.2 g potassium sorbate and stirred very well with help magnetic stirrer. The mixture was kept overnight after which 50 mL of distilled water was added. The stirring continued with addition of 10 mL and 5 mL of propylene glycol and ethanol respectively. Two to three drops of 10% of 0.1M NaOH and stirred until the gel solutions formed is at pH 7.0

Table. 1: Functions of Various Ingredients

Ingredients	Function
Carbopol 940 (Polymer)	Gelling Agent
10% Sodium Hydroxide	Neutralizer/pH Adjustor
Potassium Sorbate	Preservative
Propylene Glycol	Solvent
Ethanol	Solvent
Distilled Water	Solvent

The gel base was loaded with *Moringa oleifera* extracts to formulate 5% and 10% herbal hydrogels according to the table below

Table 2: Formulation of Herbal Hydrogel containing *Moringa oleifera* Extracts

Ingredient	HG1 (5%)	HG2 (10%)
***M. oleifera* extract**	5g	10g
Gel Base	95g	90g
Total	100g	100g

Antimicrobial Properties of the Herbal Hydrogel.

Microbiological Isolates

Pure culture of the isolates used in this study were obtained from Ahmadu Bello University Teaching Hospital, Shika, Kaduna state Nigeria and preserved in McCartney bottles with slant preparation of nutrient agar to maintain their growth. The microbial isolates used in this work were two gram positive bacteria: *Streptococcus pyogens, Staphylococcus aureus,* one gram negative bacteria: *Pseudomonas aeruginosa* and *Candida albicans.*

Antibacterial Assay:

Zone of inhibition

Antibacterial activity of the herbal hydrogel containing *Moringa oleifera* extracts was tested using the disc diffusion method described by Collin *et al.* (2004).

Sterile 6 mm disc Whatmann number 1 filter paper disc were impregnated with varying concentrations of the herbal hydrogel 400, 200, 100 and 50 mg/mL.

The bacterial cultures were inoculated on Nutrient Broth (Oxoid) and incubated for 24 h at 37°C. Adequate amounts of nutrient Agar (Oxoid), which was prepared according to manufacturer's instructions, were applied into petri dish and allowed to solidify under aseptic conditions. The bacterial cultures were adjusted to match the McFarland turbidity standard of 0.5 cfu/mL. The test microorganisms, *Streptococcus pyogens, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans,* were inoculated with a sterile swab on the surface of the solidified nutrient agar medium in Petri dish. The agar plates inoculated with the test microorganisms were incubated for 1 hour before placing the herbal hydrogel impregnated paper disc on the Petri dish. The bacterial Petri dishes were incubated at 37°C for 24 h. Zones of inhibition were observed in all Petri dishes and the diameter of these zones were recorded in millimeters. All Petri dishes were observed for zones of inhibition and the diameter of these zones were measured in millimeters. All tests were performed under sterile conditions in a biosafety cabinet.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of the herbal hydrogel was determined by the method described by Collin *et al*. (2004). Serial dilutions containing 300, 200, 100, 50 and 25mg/mL of the herbal hydrogel were prepared and then transferred to the broth in micro tube. Before inoculation of the test organisms, the bacterial or yeast strains (*Candida albicans*) were adjusted to 0.5 McFarland in diluted nutrient Broth (Oxoid). The micro tubes were incubated at 37°C for 48 h. The MIC values of the extracts were defined as the lowest concentration that showed no growth (turbidity).

Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) was determined by inoculating samples from clear test tubes showing no growth onto nutrient Agar petri dishes. MBC was defined as the lowest concentration yielding no growth subculture. In this work, Ciprofloxacin and clotrimazole which are standard antibacterial and antifungal agents respectively were used to serve as positive control while sterilized distilled water was used as negative control (Khan *et al*., 2001).

DPPH Radical Scavenging Activity of the Herbal Hydrogel

The formulated herbal hydrogel was subjected to antioxidant

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ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
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activity. Method of (Rahman *et al*., 2015) was adopted for the determination of the antioxidant activity of the herbal hydrogel with slight modification. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of herbal hydrogel in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A blank solution was also prepared and used as control. The absorbance of the mixture and control was measured spectrophotometrically at 517 nm. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\%\ \text{DPPH radical scavenging activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} * 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control, and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample.

RESULTS AND DISCUSSION

Total polyphenols and total flavonoids content of the hydroalcoholic extract of *Moringa oleifera* were quantitatively determined and the results were presented in Table 3. From the result, the concentration of phenolics compounds is slightly higher than flavonoids in the hydroalcoholic extract. These compounds were believed to possess numerous biological activities and were reported to exhibit significant radical scavenging activity as well as a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic properties (Saeed *et al.*, 2012). Earlier study by Nascimento (2017) reported the total phenolic content of 170.07 mg GAE/g from the ethanol extract of *M. oleifera* from Brazil.

Table 3: Quantitative Phytochemical Analysis of *M. oleifera* Hydroalcoholic Extract

Phytochemicals (mg GAE/g of Extract)	Concentration
Total Phenolic Contents	288.59±34.18
Total Flavonoid Contents	242.19±32.40

The formulated herbal hydrogel was analyzed for the antioxidant properties and the radical scavenging activity is presented in Fig. 1. The result shows that formulation HG2 possesses higher antioxidant activity than formulation HG1. A very large difference between the two formulations was observed at concentration 25mg/mL, where the radical scavenging activity of HG2 triples that of HG1. The antioxidant activity of plant extracts could be attributed to the presence of polyphenol compounds in the plant. These phenolic compounds could help in scavenging reactive oxygen species (ROS) by inhibiting the initiation or propagation of the oxidative chain reaction that produces them thereby delaying the processes of cellular damage, senescence processes and metabolic disorders associated with the presence of these reactive free radicals (Calvo *et al*., 2022). Ali *et al*. (2022) reported hydrogel loaded with hydro-alcoholic extract of *Moringa oleifera* seed to possess antioxidant activity.

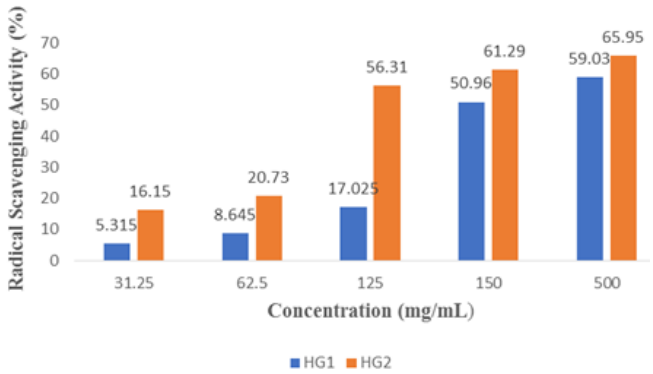

Fig. 1: Antioxidant Activities of the Formulated Herbal Hydrogels

The formulated hydrogels containing *M. oleifera* extract showed various levels of antimicrobial properties against common disease-causing pathogens; *P. aeruginosa, C. albicans*, *S. pyogens* and *S. aureus*. Zones of inhibitions of the hydrogels at different concentrations are presented in Table 4 below. From the result of the zones of inhibitions obtained, formulation HG1(5%) was found to exhibit high antimicrobial activity against *P. aeruginosa* and *S. pyogens* than formulation HG2 (10%) herbal hydrogel across all the concentrations. On the other hand, formulation HG2 was found to exhibit high antimicrobial activity against *C. albicans* and *S. aureus* than HG2. Both HG1 and HG2 showed considerable moderate zones of inhibition when compared with the standard antifungal (clotrimazole) and antibacterial (ciprofloxacin) used in this study to serve as positive controls.

The observed antimicrobial activities of the herbal hydrogels can be attributed to the level of bioactive phytochemicals such as alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, tannins, triterpenes, sterols, saponins, and some other secondary metabolites presence in the *M. oleifera* leaves (Atef *et al*., 2019). Studies carried out by Dzotam *et al*. (2016) reported the methanol extract of *M. oleifera* leaves to show different inhibition patterns against various bacterial strains including *E. coli*, *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa*. Similar antibacterial properties of aqueous and methanol extracts of *Matricaria chamomilla* against *P. aeruginosa* and *S. aureus* was reported by Abdelgadir (2016).

Table 4: Antimicrobial Activities of the Formulated Herbal Hydrogels

	Zone of Inhibition (mm)								
	400mg/mL		200mg/mL		100mg/mL		50mg/mL		Standard
Microorganism	HG1	HG2	HG1	HG2	HG1	HG2	HG1	HG2	Drug
P. aeruginosa	22	13	17	11	12	-	9	-	29
C. albicans	19	20	17	15	10	13	-	10	20
S. pyogens	20	10	15	8	10	25	-	-	25
S. aureus	15	18	9	14	-	12	-	8	24

HG1 = hydrogel containing 5% of *M. oleifera extract*, HG2 = hydrogel containing 5% *M. oleifera* extract

MIC and MBC of the herbal hydrogels were determined against all the tested microorganisms and the results were presented in tables 5 and 6 respectively. Minimum inhibitory concentrations (MICs) are the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism while minimum bactericidal

concentrations (MBCs) are the lowest concentrations of antimicrobial agent that will prevent the growth of an organism. From the results, it was found that hydrogel HG1 and HG2 exhibited highest MIC and MBC against *S. aureus* and *P. aeruginosa* respectively. The lowest MIC and MBC of 50mg/ml were observed for HG2 against *C. albicans* and *S. pyogens*.

Table 5: Minimum Inhibitory Concentration (MIC) of the Formulated Herbal Hydrogels

	Concentrations of the herbal hydrogel against tested microorganisms											
Microorganism	**300mg/ml**		**200 mg/ml**		**100 mg/ml**		**50 mg/ml**		**25 mg/ml**		**MIC**	
	HG1	**GH2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**
P. aeruginosa	-	-	-	+	-	+	-	++	++	+++	50 mg/mL	300 mg/mL
C. albicans	-	-	-	-	-	-	++	-	++	++	100 mg/mL	50 mg/mL
S. pyogens	-	-	-	-	+	-	++	-	++	+	200 mg/mL	50 mg/mL
S. aureus	-	-	-	-	+	+	++	+	+++	++	200 mg/mL	200 mg/mL

Antimicrobial (MIC/MBC); Key: - = No Growth (No Turbidity), + = Low Growth, ++ = Moderate Growth, +++ = High Grow

Table 6: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the Formulated Herbal Hydrogels

	Concentrations of the herbal hydrogel against tested microorganisms											
Microorganism	**300mg/ml**		**200 mg/ml**		**100 mg/ml**		**50 mg/ml**		**25 mg/ml**		**MBC/MFC**	
	HG1	**GH2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**	**HG1**	**HG2**
P. aeruginosa	-	-	-	+	-	+++	++	+++	++	+++	100 mg/mL	300 mg/mL
C. albicans	-	-	-	-	-	-	++	-	++	+	200 mg/mL	50 mg/mL
S. pyogens	-	-	-	-	+	-	++	+	++	++	200 mg/mL	50 mg/mL
S. aureus	-	-	+	-	++	+	++	++	+++	++	300 mg/mL	200 mg/mL

Antimicrobial (MIC/MBC); Key: - = No Growth (No Turbidity), + = Low Growth, ++ = Moderate Growth, +++ = High Growth

Conclusion

Preliminary studies on the antimicrobial and antioxidant activities of herbal hydrogel loaded with hydroalcoholic leaf extract of Moringa oleifera was performed. Hydrogels are excellent drug carriers that ensure controlled release of active ingredients of the rugs. In this work, we have successfully formulated herbal hydrogels containing 5% and 10 % of *Moringa oleifera* extract and compared their antioxidant properties and in vitro antimicrobial activities against common disease-causing microorganisms such as *S. aureus*, *P. aeruginosa, C. albicans* and *S. pyogens.* Results obtained show the activities of the hydrogel to be concentration dependent. While most of the microorganisms where sensitive against formulation with higher percentage (10%) of the extract, others were sensitive against formulation with the lower percentage (5%) of the extract. This indicates that herbal hydrogel containing *Moringa leaf* extract can be used for the treatment of infection caused by these microorganisms if properly standardized. An extensive research work is recommended in order to evaluate the *in vivo* activities against specific diseases caused by these microorganisms as well as to investigate the cytotoxicity of herbal hydrogel loaded with *M. oleifera* leaf extracts

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