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Assessment of A Nanoemulsion Gel Made from Moringa Seed Oil using Anticollegenase

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Annotation: This study's goal was to create and assess a moringa seed oil (MSO) nanoemulsion gel utilizing the high-energy emulsification technique. Techniques: By comparing the concentrations of surfactant (tween 80) and cosurfactant (sorbitol) with variations in the concentration of moringa seed oil, a nanoemulsion gel was created utilizing the high-energy emulsification method. Centrifugation testing, viscosity, pH, organoleptic observation (odor, color, clarity, and phase separation), and particle size measurement throughout a 12-week storage period at room temperature are all used to assess the stability of the nanoemulsion gel formulation.

Introduction: -

In many underdeveloped nations, including India, medicinal plants are frequently employed as the main source of treatment. Instead of using contemporary medication, many people rely on these botanicals. For generations, a variety of plant parts, including leaves, roots, stems, and flowers, have been utilized to treat various ailments. According to estimates from the World Health Organization (WHO), more than 80% of people on the planet rely on medicinal plants for medical care.

These plants' natural chemical constituents, referred to as phytochemicals, are what give them their therapeutic qualities. These consist of flavonoids, alkaloids, tannins, and saponins, which can increase therapeutic benefits either separately or in combination. These substances can improve the body's ability to absorb and metabolize medications.

Moringa oleifera is rich in vitamins, proteins, and other vital nutrients.

Moringa has the ability to prevent and treat a number of ailments due to its high concentration of beneficial chemicals. Moringa may help control diabetes, obesity, and some forms of cancer, according to research. Strong antibacterial, anti-inflammatory, and antioxidant qualities are also present.

Hydrogels are unique materials that resemble gels and have a high water absorption capacity. They are extensively employed in medicine for skin treatments, wound healing, and drug administration. By regulating the release of medications, hydrogels can improve therapy efficacy while lowering adverse effects. Because of these advantages, scientists are looking for ways to mix gels with moringa extract to create more effective therapies for skin conditions. The "tree of life" is another name for Moringa oleifera.

Fig No. 1 Moringa Oleifera

Literature Review:

This study examines the ability of methanolic Moringa oleifera extract to cure wounds infected with P. aeruginosa or MRSA in diabetic rats. Its chemical components were determined by phytochemical and GC-MS analyses, and tests conducted in vitro on hacat cell lines revealed elevated expression of TGF-β1 and VEGF. When used as an ointment, the extract improved collagen production, wound contraction, and antioxidant enzyme activity in diabetic rats infected with MRSA, but it was less successful against P. aeruginosa. These results imply that M. Oleifera extract aids in the healing of MRSA-infected wounds, but more investigation is needed to determine its wider antimicrobial activity, particularly against P. Aeruginosa.

Diabetes patients' delayed wound healing is a serious issue on a global scale.

The two main things that prevent diabetes patients' wounds from healing are hyperglycemia and elevated free radical levels. Because plant extracts are a rich source of polyphenols, they can effectively promote wound healing. The stability of polyphenols in plant materials is significantly impacted by drying temperature and extraction solvent.

To guarantee the effectiveness of the finished product, the extraction procedure must be optimized.
In order to achieve this, the current study investigated the effects of drying temperature and solvents on the polyphenolic composition and diabetic wound healing capacity of Moringa oleifera leaves.

Materials And Methods:-

Chemicals and Reagents

They include but are not limited to Mueller-Hinton agar (MHA) (LS Biotech, UK), Sabouraud

dextrose agar (SDA) (LS Biotech, UK), distilled water procured from Anslem Chemicals, Nigeria; M. oleifera oil processed in our laboratory, soybean oil (Aromachem, UK), glycerylmonooleate (Sigma Aldrich, USA), Peceol® and Labrasol® were gifts from Gattefosse, Saint-Priest, France, Kolliphor® ELP was provided by BASF, Ludwigshafen, Germany, Tween 80 (Sigma Aldrich, USA), polyethylene glycol (PEG) 400 (Sigma Aldrich, USA), petroleum ether (Loba Chemie, India), sodium hydroxide (GH Tech, China), ammoniumsulphate (GH Tech, China), dimethylsulphoxide (Molychem, India).

Collection and Identification of M. Oleifera Seeds

The plant, which was cultivated on a farm in Katsina State, North-West Nigeria, was collected and preserved in sealed pouches. It was then cleansed to eliminate grit, desiccated leaves, and other debris. A plant taxonomist from the Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, identified the seed.

Processing and Defatting of Seeds

The seeds were removed from their shells using a seed processor, and the desiccated nuts were ground into fine granules using a milling machine (Qasa Blender, Qlink Group, China) and sieved to obtain uniform-sized powders with a stainless-steel laboratory sieve of 1mm. The M. oleifera seed powders were defatted by soaking in some quantity of petroleum ether (boiling point 60-90 °C) for 10 runs at 75 for 4h. The defatted M. oleifera seed powders were dried to remove residual solvent (Nebolisa et al., 2023).

Table 1. Nanoemulsion constituents

Test samples	Glyceryl monooleate(ml)	Tween 80 (ml)	Polyethylene glycol (ml)	*M. oleifera* protein (g)	Water q.s.p. (ml)
MNE1	20	2	1	0.1	100
MNE2	20	2	1	0.3	100
MNE3	20	2	1	0.5	100
MNE4	20	2	1	1	100
MNE5	20	2	1	---	100

Preparation of M. Oleifera Seed Protein Nanoemulsions:-

Through spontaneous emulsification, the nanoemulsions were prepared. The organic phase consisting of oil (Pecol® - Glyceryl monooleate, 15%), surfactant (Tween 80), and MOSP homogenized to form a uniform phase, were introduced into the aqueous phase containing distilled water and a cosurfactant (PEG 400, 1%), under magnetic stirring. In order to achieve a state of balance the mixture was agitated using magnetic stirring for a duration of 30 minutes (Araújo et al., 2011). A unique series of formulations was created, consisting of one placebo and the remaining ones containing different quantities of the protein. The formulation design was to study the effect of various concentrations of MOSP.

Table 2. *M. oleifera* seed protein solubility in oils, surfactants, and co-surfactants

Solubility in oils		Solubility in surfactants		Solubility in co-surfactants	
Oils	Protein (mg)	Surfactants	Protein (mg)	Co-surfactants	Protein (mg)
Soybean oil	160 ± 11	Kolliphor® ELP	186 ± 11	Glycerol	181 ± 10
Peceol®	301 ± 5	Tween® 80	276 ± 16	Propylene glycol	101 ± 13
M. oleifera oil	177 ± 6	Labrasol®	151 ± 9	PEG 400	270 ± 15

Results And Discussion:-

The M. oleifera protein's solubility in oil, surfactant, and cosurfactant helps choose an appropriate vehicle for nanoemulsion formulation. In the oil solubility test, Peceol® (glyceryl monooleate) had the highest solubilization for M. oleifera protein, as shown in Table 2. The M. oleifera protein was less soluble in other oils. Peceol was therefore chosen for the nanoemulsion's formulation.

When a biuret reagent was present, the test solution's hue changed from blue to violet-purplish. Copper sulfate, sodium hydroxide, and sodium potassium tartrate make up the biuret complex, which serves to sustain the cupric ion in the basic medium. The generation of complex ions between cupric ions and peptide links in the proteins of M. oleifera under alkaline circumstances is responsible for the observed hue (Nielsen, 2017).

After being heated in a water bath for five minutes, the test solution took on a deep blue hue when ninhydrin reagent was present. Ninhydrin dissolved in either ethanol or acetone makes up the ninhydrin reagent. A dark blue color is created by heating a mixture of water-based solutions of M. oleifera protein, which is made up of amine residues in the side strands of linear or aliphaticaromatic amine. The process begins with the amino acid being oxidatively deaminated, which produces ammonia and reduces ninhydrin to hydrindantin.

A blue hue is produced when hydrindantin and ammonia combine to make dietohydrindylidenediketohydrindamine (Friedman, 2004; Yemm et al., 1955).

Conclusion:-

Glyceryl monooleate, polysorbate 80, and PEG 400 are examples of biodegradable polymers that can be used as nanoemulsion components. Investigating plant peptides as antibacterial agents is advantageous and environmentally favorable. By reducing the exposure of the protein's three-dimensional structure throughout formulation, storage, and transportation, Polysorbate 80 assisted in maintaining the biological activity of M. oleifera protein. Plant proteins are supported by PEG, which decreases protein aggregation and proteolysis and lengthens the shelf life of proteins.

The nanoemulsion had a high dynamic viscosity and an acidic pH. The time-kill test revealed modest biocidal activity, however the antimicrobial and minimum inhibitory concentration studies indicated strong antimicrobial efficacy against P. aeruginosa and S. aureus. The molecular mechanism by which M. oleifera nanoemulsion showed decreased efficacy against bacteria requires more investigation. More research should be done to see whether altering the lipids could enhance antibacterial action.

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