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FORMULATION AND EVALUATION OF HERBOSOMES OF *ADENOSTEMMA LAVENIA* AND ITS ANTI MICROBIAL ACTIVITY

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ABSTRACT

Herbosomes, a novel lipid-based drug delivery system, were formulated using *Adenostemma lavenia* extract to enhance its therapeutic potential. The study involved the preparation of herbosomes by the thin-film hydration method and their subsequent evaluation for various parameters. Physicochemical characterization, including particle size, zeta potential, entrapment efficiency, and morphology, was performed to assess the quality of the formulated herbosomes. The resulting formulations were found to be porous, smooth, and spherical. The particle size and zeta potential were determined using the Malvern Zeta sizer, with the average particle size for formulations F1 and F5 being 158.8 nm to 551.3 nm. The antimicrobial activity of *Adenostemma lavenia* herbosomes was evaluated against microbial strain using standard methods. As the concentration of the herbosomes increases, there is a noticeable increase in the zone of inhibition, indicating enhanced antimicrobial activity against *E. Coli*.The results indicated that the formulated herbosomes exhibited promising antimicrobial activity compared to the free extract. This study demonstrates the potential of *Adenostemma lavenia* herbosomes as effective antimicrobial agents, suggesting their potential application in pharmaceutical formulations.

Keywords:*Adenostemma lavenia*, herbosomes, antimicrobial activity, lipid-based drug delivery, thin-film hydration, phytoconstituents.

1. INTRODUCTION

The World Health Organisation (WHO) defines herbal medicines as finished, labelled pharmaceutical preparations containing an active ingredient from the plant, aerial or underground, or other plant material or mixes.[1] To make herbal medication, the bioactive extract should be standardised based on the active ingredient and submitted to stringent safety tests. The active ingredient is directly related to the bioactivity or efficacy of herbal medication.[2]Herbal medication offers good efficacy and only a few adverse consequences when used properly.[3]

Adenostemma lavenia(L.) O. Kuntze typically grows uncontrolled, sometimes even being regarded as a weed. *A. lavenia*, sometimes called sticky daisy, is a tropical Asian plant that spreads widely. Although practically all Indonesian provinces have *A. lavenia*, cultivation of the plant is not common. It is referred to as legetan warak in Java.[4]Backer and Bakhuizen van den Brink (1965) identified *A. lavenia* and other plant species found in Java. *A. lavenia* was characterised as having a glandular-hairy or subglabrous stem, elliptical, obtuse, or acute apex, and dentate or serrate leaves. It also thrives in locations that are somewhat shaded, humid, woods, brushwood, ditches, and beside roads.A perennial herb that is commonly found in Asia's tropical regions, *Adenostemma lavenia* (L.) O. Kuntze is a member of the Asteraceae family. The Pacific Islands have long utilised this plant as a medicinal herb to treat skin wounds, fever, hepatitis, pneumonia, and lung congestion.[5] In mice and RAW 264.7 cells exposed with lipopolysaccharide (LPS), a recent study demonstrated the anti-inflammatory properties of *A. lavenia* extract.[6] Furthermore, B16F10 cells exhibit antimelanogenic actions of this plant's leaf extract, and young mice's hair pigmentation is suppressed.[7,8]Due to its significant potential, scientists have tried to develop many formulationsbut poor lipophilicity of extract, resulting in the terms of low bioavailability.[9]By keeping in mind these things, we have tried todevelop the Herbosome of *A. lavenia* extract inthe currentdiscussedstudy.

The newest technique, particularly for the administration of herbal drugs, is called herbosome. The word herbosome is derived from the words herbs, which signify plants, and some, which means something that, resembles cells. According to research in this area, herbosomes are sufficiently capable of delivering the medication inside the body in a way that makes it bioavailable.[10] The phytocompound's lipid solubility and, eventually, permeability are improved when phospholipids are added.[11]As per literature search and our present knowledge, this is the first attempt made on to prepare Herbosome using *Adenostemma lavenia*.

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Adenostemma lavenia*(300 gm) was collected. After cleaning, plant parts (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Adenostemma lavenia*was authenticated by a plant taxonomist in order to confirm its identity and purity.

2.2 Extraction

In the current investigation, plant material was extracted utilizing the continuous hot percolation method with Soxhlet equipment. Powdered *Adenostemma lavenia* was inserted in a thimble of the soxhlet device. Soxhlation was performed at 60°C with petroleum ether as the non-polar solvent. The exhausted plant material (marc) was dried and then re-extracted with mzx ethanol solvent. For each solvent, soxhlation was maintained until no visual color change was noticed in the siphon tube, and extraction was confirmed by the absence of any residual

solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type). Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use[12].

2.3 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests[13].

2.4 Formulation of extract loaded herbosomes formulation

Herbosomes are basically herbal-liposomes specially designed to enhance the bioavailability of the herbals so that they have targeted site of action. In this study, we have designed herbosome by using conventional method of preparation i.e., thin film hydration (TFH) method of liposomes describe with few modifications. Briefly soya lecithin and cholesterol were dissolved in different concentration in chloroform-methanol (1:1) and 200 mg *Adenostemma lavenia*was added in the solution, then the mixture was evaporated in a rotary evaporator. The thin film formed in the round-bottomed flask was hydrated by adding phosphate buffer 7.4. The suspension was stirred by magnetic stirring for 30 min and then sonicated for 5 to 25 minutes**.** The various formulation variable considered in this research have been presented in Table 1. Phytosome was then successfully collected in vessels and used for further drug development[14].

Table 1: Composition of herbosomes formulation

Ingredients	Formulations				
	F1	F2	F3	F4	F5
Extract (mg)	200	200	200	200	200
Soya-Lacithin (mg)	50	100	150	200	250
Cholesterol (mg)	15	15	15	15	15
Sonication Time (min)	5	10	15	20	25
chloroform-methanol (1:1) ml	20	20	20	20	20
phosphate buffer 7.4	qs	qs	qs	Qs	qs

2.5 Characterization of herbosomes

2.5.1Particle size and Zeta potential

The particle size is one of the most important parameter for the characterization of herbosomes. The size of herbosomes was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the phytosome was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements. The data is documented in Table 4[15-16].

2.5.2 Scanning Electron Microscopic (SEM)

The morphological properties of the extract-loaded herbosomes were obtained using the electron beam from a scanning electron microscope. The herbosomes were coated with a thin layer (2-20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater in vacuum. The pretreatment specimen was then attacked with an electron beam, which resulted in the creation of secondary electrons known as augers. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography[17].

2.6 Anti-microbial activity

2.6.1 Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

2.6.2 Well Diffusion Assay

The bacterial suspension of *E. coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate[18]. The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the Phytosomes (0.5, 1, 1.5 and 2mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. Following incubation, plates were examined for the creation of a clear zone around the well, which corresponded to the antibacterial activity of the tested drugs. The zone of inhibition (ZOI) was examined and measured in millimetres. Zones were measured to the closest millimeter with a ruler held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Adenostemma lavenia*is shown in Table: 2

Table 2: Percentage Yield of crude extracts of *Adenostemma lavenia*extract

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Adenostemma lavenia*	Pet ether	300	2.36	0.786%
2		Methanol	279	6.10	2.26%

3.2 Preliminary Phytochemical study

Table3: Phytochemical testing of extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	**Methanolic extract**
Alkaloids		
Dragendroff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Absent	Present
Glycoside		
Borntrager test	Present	Present
Legal's test	Present	Present
Killer-Killiani test	Present	Present
Carbohydrates		
Molish's test	Absent	Present
Fehling's test	Absent	Present
Benedict's test	Absent	Present
Barfoed's test	Absent	Present
Proteins and Amino Acids		
Biuret test	Present	Absent
Flavonoids		
Alkaline reagent test	Absent	Present
Lead Acetate test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Saponin		
Foam test	Present	Absent
Test for Triterpenoids and Steroids		
Salkowski's test	Present	Absent
Libbermann-Burchard's test	Present	Absent

3.3 Characterization of Herbosomes

3.3.1 Particle Size and Zeta potential

Table 4: Particle size and Zeta potential

Formulation code	Particle size (nm)	PI Value	Zeta potential
F1	551.3 nm	4.181	-11.9 mV
F2	**158.8 nm**	**0.369**	**-49.7 mV**
F3	164.5 nm	0.398	-12.4 mV
F4	173.3 nm	3.260	-29.5.mV
F5	166.5 nm	0.295	-18.0 mV

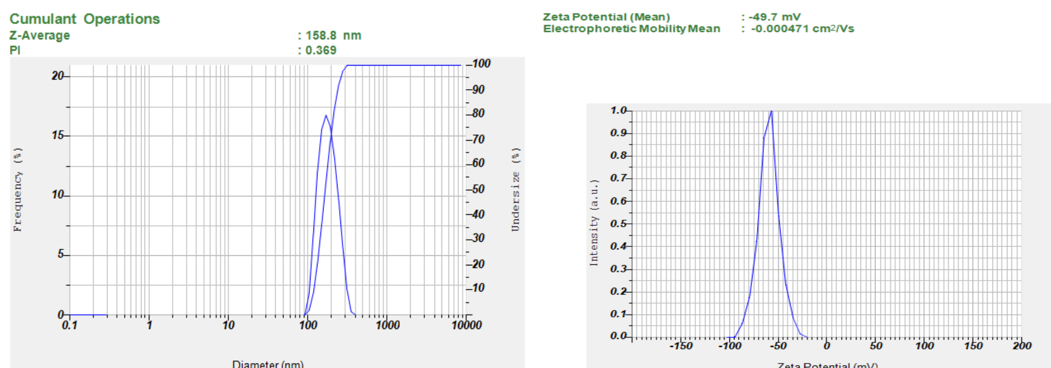

Figure1: Particle size and Zeta potential (F2)

The particle size is one of the most important parameter for the characterization of Herbosomes. The average particle size of the prepared extract loaded herbosomes was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of extract loaded herbosomes was found to be range 158.8 nm to 551.3 nm.Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. Herbosomes with zeta potential value greater than +25 mV or less than -25 mV typically have high degrees of stability. If the particles in Herbosomes have a high positive zeta potential, they will reject one another and have no tendency to come together. However, if the particles have low zeta potential values, there will be no force to keep them from clumping together and flocculating for herbosomes. Zeta potential of all formulations was found to be range -11.9 mV to -49.7 mV with peak area of 100% intensity. These values indicate that the formulated Herbosomes are stable.

3.3.2 Scanning electron microscope (SEM)

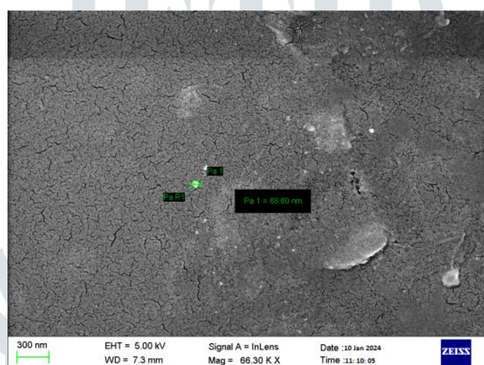

Figure2: Scanning electron microscope (SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared Herbosomes. Herbosomes were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared herbosomes at 66.30 kx magnification showed that the herbosomes were porous with a smooth surface morphology and spherical shape. The porous nature of herbosomes was clearly observed in the SEM images.

3.4 Anti-microbial activity

Table 5: Antimicrobial activity of Herbosome against *E.coli*

Sample name	Zone of Inhibition (mm)
Herbosomes (0.5mg/ml)	7 mm
Herbosomes (1mg/ml)	10 mm
Herbosomes (1.5mg/ml)	12 mm
Herbosomes (2mg/ml)	15 mm

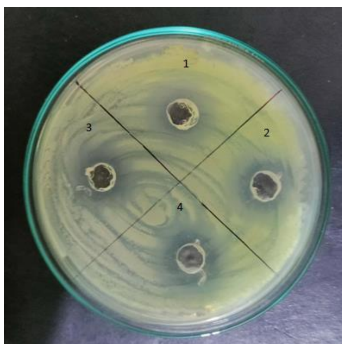

Figure3: Antimicrobial activity against *E. coli*

The results of antimicrobial activity testing against *E. Coli* for different concentrations of herbosomes are presented in Table 5 and illustrated in Figure 3. As the concentration of the herbosomes increases from 0.5 mg/ml to 2 mg/ml, there is a noticeable increase in the zone of inhibition, indicating enhanced antimicrobial activity against *E. Coli*.At a concentration of 0.5 mg/ml, the herbosomes exhibited a modest zone of inhibition of 7 mm. However, at higher concentrations of 1 mg/ml and 2 mg/ml, the zones of inhibition increased significantly to 10 mm and 15 mm respectively. This concentration-dependent increase in antimicrobial activity suggests that the herbosomeseffectively delivers the antimicrobial activity, likely *Adenostemma lavenia*extract, to the target microorganism.The observed antimicrobial activity of the herbosomes against *E. Coli* is promising and suggests its potential application as antimicrobial agent. The increasing zone of inhibition with higher concentrations indicates a dose-response relationship, reinforcing the effectiveness of the herbosomes.

4. CONCLUSION

The study reveals the potential of *Adenostemma lavenia* extract's phytochemicals, including alkaloids, carbohydrates, flavonoids, glycosides, and phenols, for enhancing bioavailability. The herbosomal formulation was tested using a thin film hydration method, and polymers were found to be suitable for the *Adenostemma lavenia* Herbosome. The resulting formulations were found to be porous, smooth, and spherical. The particle size and zeta potential were determined using the Malvern Zeta sizer, with the average particle size for formulations F1 and F5 being 158.8 nm to 551.3 nm. The study also suggests that the herbosome-based drug delivery approach could improve the therapeutic efficacy of phytochemicals by improving their absorption and bioavailability by altering their physicochemical and release properties. Further studies are needed to further explore the herbosomal formulation of *Adenostemma lavenia* extract.

5. REFERENCES

1. Parveen A, Parveen B, Parveen R, Ahmad S. Challenges and guidelines for clinical trial of herbal drugs. Journal of pharmacy and bioallied sciences. 2015 Oct 1;7(4):329-33.
2. Khan MS, Ahmad I. Herbal medicine: current trends and future prospects. InNew look to phytomedicine 2019 Jan 1 (pp. 3-13). Academic Press.
3. OCKTARINI R, PRASETYO DH, SJARIFAH I. Effect of herbal extract of anting-anting (Acalypha australis) on blood glucose level of Balb/C mice with induction of Streptozotocin. Asian Journal of Natural Product Biochemistry. 2011 Feb 17;9(1):12-6.

4. Batubara I, Komariah K, Sandrawati A, Nurcholis W. Genotype selection for phytochemical content and pharmacological activities in ethanol extracts of fifteen types of Orthosiphon aristatus (Blume) Miq. leaves using chemometric analysis. Scientific Reports. 2020 Dec 1;10(1):20945.
5. Cheng PC, Hufford CD, Doorenbos NJ. Isolation of 11-hydroxyated kauranic acids from Adenostemma lavenia. Journal of natural products. 1979 Mar 1;42(2):183-6.
6. Chen JJ, Deng JS, Huang CC, Li PY, Liang YC, Chou CY, Huang GJ. p-Coumaric-acid-containing Adenostemma lavenia ameliorates acute lung injury by activating AMPK/Nrf2/HO-1 signaling and improving the anti-oxidant response. The American Journal of Chinese Medicine. 2019 Oct 24;47(07):1483-506.
7. Budiarti E, Batubara I, Ilmiawati A. The potency of Asteraceae plants extracts as antioxidant and antiglycation agent. J. Jamu Indones. 2019;4:109-17.
8. Hamamoto A, Isogai R, Maeda M, Hayazaki M, Horiyama E, Takashima S, Koketsu M, Takemori H. The high content of Ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid in Adenostemma lavenia (L.) O. Kuntze leaf extract: With preliminary in vivo assays. Foods. 2020 Jan 9;9(1):73.
9. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr. Journal of ethnopharmacology. 2005 Jan 4;96(1-2):159-63.
10. Vora A, Londhe V, Pandita N. Herbosomes enhance the in vivo antioxidant activity and bioavailability of punicalagins from standardized pomegranate extract. Journal of functional foods. 2015 Jan 1;12:540-8.
11. Dewan N, Dasgupta D, Pandit S, Ahmed P. Review on-Herbosomes, A new arena for drug delivery. Journal of Pharmacognosy and Phytochemistry. 2016;5(4):104-8.
12. Baidya B, Gupta SK, Mukherjee T. An extraction-based verification methodology for MEMS. Journal of Microelectromechanical Systems. 2002 Feb; 11(1):2-11.
13. Kokate SD, Satav JG, Nair CK. Influence of natural pesticides on biochemical parameters in mosquito Culex pipiens quinquefasciatus Say. Indian Journal of Toxicology. 2000;7(1):35-8.
14. BalaYadav R, Pathak DP, Varshney R, Arora R. Design and optimization of a novel herbosomal-loaded PEG–poloxamer topical formulation for the treatment of cold injuries: a quality-by-design approach. Drug Delivery and Translational Research. 2022 Nov;12(11):2793-823.
15. Singh KK, Vingkar SK. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. International Journal of Pharmaceutics. 2008 Jan 22;347(1-2):136-43.
16. Đorđević SM, Cekić ND, Savić MM, Isailović TM, Ranđelović DV, Marković BD, Savić SR, Stamenić TT, Daniels R, Savić SD. Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: Design, characterization and in vivo pharmacokinetic evaluation. International journal of pharmaceutics. 2015 Sep 30;493(1-2):40-54.

17. Anwer MK, Mohammad M, Ezzeldin E, Fatima F, Alalaiwe A, Iqbal M. Preparation of sustained release apremilast-loaded PLGA nanoparticles: In vitro characterization and in vivo pharmacokinetic study in rats. International journal of nanomedicine. 2019 Mar 1:1587-95.
18. Mohammadi-Sichani M, Karbasizadeh V, Aghai F, Mofid MR. Effect of different extracts of Stevia rebaudiana leaves on Streptococcus mutans growth. J Med Plants Res. 2012 Aug 22;6(32):4731-4.