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Effect of Organic Solvents in the Preparation of Single Aged Garlic Transfersomes and Their Phytochemical Activities

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ABSTRACT. Single Aged Garlic (SAG), a fermented product from a single garlic, has been extensively studied for its health benefits due to its allicin content. To enhance SAG's drug delivery capabilities, this study aimed to characterize transfersome formulations containing SAG, investigating their phytochemical activities and the effects of different absolute solvents. Transfersome formulations, consisting of soy-phospholipid and either Span-60 (T1) or Tween-80 (T2), were prepared using absolute ethanol (EA) or a chloroform-methanol mixture (CM). Characterization included particle size, polydispersity index (PDI), and zeta potential. Phytochemical tests assessed antioxidant activity, total phenolic content, and total flavonoid content. Results showed that T2-CM formulations exhibited the best PDI (0.372 ± 0.022), smallest particle size (T1-CM: 84.333 ± 1.762 nm), and lowest zeta potential (T2-EA: -25.667 ± 0.666 mV). Additionally, T1-CM and T2-CM formulations demonstrated superior antioxidant, flavonoid, and phenolic content compared to T1-EA and T2-EA. Transfersomes formulated with organic solvents like absolute ethanol, methanol, and chloroform exhibit promising characteristics and can effectively protect the antioxidant compounds, flavonoids, and phenols present in SAG extracts. These solvents, known for their ability to dissolve polar and nonpolar compounds, facilitate the formation of stable, well-characterized transfersomes. These findings suggest that transfersomes prepared with chloroform-methanol mixtures are more promising for SAG delivery.

Key words: Organic solvents, Phytochemicals, Single aged garlic, Transfersomes

INTRODUCTION

Garlic (*Allium sativum* L.) is one type of plant that has been widely used both in the field of food and health. Garlic bulbs contain allicin and sulfur amino acid alliin. Studies have shown that garlic has various medical effects, such as antibacterial, antifungal, antihypertensive, and anticancer properties. Garlic also has antioxidant properties (Capasso, 2013; Lee & Gao, 2012; Sanjay et al., 2018). There are several varieties of garlic, one of which is single garlic (*A. sativum* var. Solo Garlic). Single garlic consists of only one clove and has the highest antioxidant activity among the three varieties of garlic. The IC_{50} value of the garlic extract using with 70% ethanol is 10.61 μg/mL, lower than that of local garlic Ciwidey (13.61 μg/mL) and imported garlic (11.32 μg/mL), indicating its stronger antioxidant activity. The ethanol extract was in the form of a thick extract (Ilmawati et al., 2017; Qadariah et al., 2020).

One form of single garlic consumption is *single aged garlic* (SAG). SAG is a fermented product of Single garlic that is brewed at 65-80°C with a humidity of 70-80% of room temperature for 21 days. SAG has a black color and light mass due to its reduced moisture content, and has an aroma and taste that is not too pungent like garlic (Lu et al., 2017). SAG antioxidants have stronger activity compared to garlic with Trolox Equivalent Antioxidant (TEAC) values of 13.3 ± 0.5 and 59.2 ± 0.8 mol/g wet, respectively (Choi et al., 2014). SAG, containing S-allycysteine, exhibits twice the antioxidant and antibacterial potency of regular garlic. Its potent antioxidant activity, attributed to elevated flavonoid and polyphenol levels from the fermentation process, effectively scavenges free radicals (Lawson & Hunsaker, 2018; Tran et al., 2018). The susceptibility of these bioactive compounds to oxidation and degradation diminishes their therapeutic efficacy. The implementation of modified

drug and dosage delivery systems for SAG is necessary. Modification of preparations in the form of delivery systems using vesicle systems such as liposomes, transfersomes and ethosomes has been reported to increase the ability of drugs to penetrate the skin (Chaurasiya et al., 2019; Sharma et al., 2014). Given the dual nature of SAG's phytochemicals, the versatile transfersome delivery system is ideal for optimizing the penetration, bioavailability, and stability of active compounds, paving the way for innovative skin care products (Mitchell et al., 2021). Transfersomes are ultra-flexible that can easily penetrate the skin and overcome the stratum corneum barrier by disrupting its lipid structure. Transfersomes can deform and pass through pores that are 5-10 times smaller than their diameter, and have high entrapment efficiency for lipophilic drugs, reaching up to 90%. Transfersomes are composed of hydrophobic and hydrophilic components, making them suitable for drugs with low deformability (Chaurasiya et al., 2019; Sachan et al., 2013).

Transfersomes consist of phospholipids such as phosphatidylcholine as vesicle-forming components, surfactants as edge activators to increase flexibility, absolute ethanol, methanol, and chloroform as solvents, and buffer solutions as hydrating media (Omar et al., 2019). Choosing the right solvent greatly affects how stable and effective the drug delivery system is when making transfersomes from SAG extract. Absolute ethanol is used due to its strong polarity, which enables it to effectively dissolve various bioactive compounds present in the SAG extract, such as flavonoids, phenolic compounds, and alkaloids (Chang et al., 2018; Okonogi et al., 2021). These bioactive molecules contribute to the therapeutic effects of the extract, and their proper dissolution is crucial for ensuring uniform distribution within the transfersomes. Additionally, absolute ethanol enhances the fluidity of the lipid bilayer, improving the vesicles' ability to penetrate biological membranes (Sharma et al., 2014; Singh & Gaikwad, 2021). Meanwhile, a combination of methanol and chloroform is utilized to dissolve specific classes of compounds that are not efficiently solubilized by ethanol alone. Methanol, being a polar solvent, is particularly effective in extracting hydrophilic substances such as certain flavonoids and phenolic acids (Jang et al., 2017). In contrast, chloroform, a non-polar solvent, facilitates the dissolution of lipophilic compounds, including phospholipids and certain terpenoids. This biphasic solvent system ensures comprehensive extraction of both hydrophilic and lipophilic constituents, optimizing the formulation and stability of transfersomes (Krishna, 2023; Rasheed et al., 2022). Absolute ethanol is more polar than the mixture of methanol and chloroform because the non-polar chloroform reduces the overall polarity of the mixture (Apsara et al., 2020; Rasheed et al., 2022). In the thin-film hydration method for making transfersomes, lipids and surfactants must be dissolved in organic solvents until homogeneous. The selected solvent must be soluble in both the lipid component and water (Apsara et al., 2020). The selection of organic solvents in the preparation of transfersomes is important. The composition of lecithin as phospholipids and surfactants is a variable that can affect the optimization of the transfersome formula (Khan et al., 2022; Surini & Djajadisastra, 2018). Some surfactants that can be used for transfersome formulations include single-chain surfactants that can disrupt the lipid bilayer and increase transfersome deformability. Examples include sodium cholate, sodium deoxycholate, span-60, span-65, span-80, tween-20, tween-60, tween-80, and dipotassium glycyrrizinate (Khan et al., 2022; Sachan et al., n.d.). Span-60 and tween-80 have high skin penetration and smaller particle size, which can increase flexibility in the lipid bilayer membrane of lecithin vesicles, allowing transfersomes to pass through pores smaller than their size spontaneously (Leonyza & Surini, 2019). This study was conducted to characterize transfersome formulas and the phytochemical activities of SAG with different absolute organic ethanol solvents and chloroform methanol mixtures. Characterization included particle size, polydispersity index, and zeta potential. Phytochemical testing included antioxidant activity using the DPPH method, total phenol content, and total flavonoid content.

EXPERIMENTAL SECTION

Materials

This research utilized the following materials: single garlic sourced from Sarangan Village, Magetan District, East Java, Indonesia, absolute ethanol (Merck, Germany), Methanol (Merck, Germany), chloroform (Merck, Germany), phospholipid (Sigma Aldrich, Germany), Tween-80 (Sigma Aldrich, Germany), Span-60 (Sigma Aldrich, Germany), 2,2-Diphenyl-1-picrylhydrazyl (Sigma Aldrich, Germany), $NaNO_3$ (Merck, Germany), $AlCl_3$ (Smart Lab, Indonesia), NaOH (Merck, Germany), Folin-Ciocalteu reagent (Merck, Germany), Na_2CO_3 (Merck, Germany), Quercetin (Sigma Aldrich), and Gallic acid (Sigma Aldrich, Germany).

The tools employed in this research included the Particle Size Analyzer (HORIBA SZ-100, Japan), Transmission Electron Microscope (TEM) (Tecnai 200 kV D2360 SuperTwin, Japan), UV-VIS spectrophotometer (Libra S11/12 Visible & UV Spectrophotometers, UK), magnetic stirrer (Thermolyne Cimarec® 2, USA), sonicator (IWAKI Ultrasonic Cleaner, Japan), analytical balance (OHAUS), Microwave Assisted Extraction (MAE) device (Microwave Reaction System SOLV Multiwave PRO, Anton Paar, Austria), rotary vacuum evaporator (Heidolph Rotary Evaporator Hei-VAP Expert, Germany), and waterbath (STUART SBS40, UK).

Extraction of Single Aged Garlic (SAG)

SAG is made by cleaning the dirt from the garlic skin and then fermenting it at 75°C for 21 days. The finished SAG is characterized by a change in color from the original white to a blackish-brown. SAG is inserted into the vessel of the Microwave Assisted Extraction (MAE) device (Microwave Reaction System SOLV Multiwave PRO, Anton Paar, Austria). SAG is peeled, crushed, and then mixed with absolute ethanol (Merck, Germany) in a 1:10 ratio in the MAE vessel. The vessel is closed, arranged in the MAE, and operated at a holding temperature of 50°C for 10 minutes with a power of 1500 W. The solvent is evaporated using a rotary vacuum evaporator (Heidolph Rotary Evaporator Hei-VAP Expert, Germany) set at a speed of 50 rpm and a temperature of 37°C. The evaporation results are heated in the oven at 40°C to remove excess solvents and obtain pure SAG extract.

Transfesome Formulation as Drug Delivery for SAG active Compounds

Transfersome preparation was carried out using a modified thin-film hydration method (Omar et al., 2019). The phospholipid component (Sigma Aldrich, Germany) weighed 0.167 g, and edge activator consisting of surfactants 0.033 g of Tween-80 (Sigma Aldrich, Germany), and Span-60 (Sigma Aldrich, Germany) were dissolved in a mixture of organic solvents chloroform and methanol (CM) (2:1, v:v) and absolute ethanol (EA) (Merck, Germany). T1 contains phospholipid and Tween-80, while T2 contains phospholipid and Span-60. The mixture was heated in a waterbath (STUART SBS40, UK) at 50 °C, stirred for 1 hour, and then evaporated. A thin film was formed after the solvent evaporation stage. The thin film was hydrated with phosphate-buffered saline (PBS) containing 0.01 g SAG. The hydration results were then sonicated (IWAKI, Japan) for 30 minutes, stirred again for 1 hour, and stored at 4 °C.

Characterization of T-SAG

Particle size characterization was performed to confirm that T-SAG particles are in the nanoscale range. The test was conducted at the Integrated Laboratory & Research Center (ILRC) of the University of Indonesia. Measurements of particle average (Z-average), polydispersity index (PDI), and zeta potential of T-SAG were carried out using a Particle Size Analyzer (PSA) (Horiba SZ 100z, Japan), with three repeated measurements.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Activity

The DPPH antioxidant test was conducted with modifications (Lestari et al., 2023). DPPH (Sigma Aldrich, Germany) was prepared at a concentration of 50 µM in methanol solvent. Transfersome samples and DPPH solution were mixed in a 1:5 (v/v) ratio and incubated for 15 minutes at room temperature in dark conditions. The absorbance of the DPPH mixture and sample was then measured using a UV-Vis spectrometer (Biochrom 12S, UK) at a wavelength of 517 nm. The resulting absorbance value was entered into Equation 1.

$$\%\text{Inhibition: } \frac{Control\ Absorbance - Sample\ absorbance}{Control\ Absorbance}\ x\ 100\% \quad (1)$$

Total Flavonoid Content (TFC)

Each 200 µL transfersome sample was added to 280 µL of dH_2O and 60 µL of 5% $NaNO_3$ (Merck, Germany) and incubated at room temperature in dark conditions for 5 minutes. After incubation, 60 µL of 10% $AlCl_3$ (Smart Lab, Indonesia) was added in dark conditions for 6 minutes. The reaction was stopped by adding 400 µL of 1 M NaOH (Merck, Germany). The TPC of each transfersome's formula was initially determined from the standard curve of Quercetin prepared range from 1 to 50 mg/L solutions of gallic acid in water. Samples that positively contain flavonoids will change color to yellow. The results were then read at 510 nm absorbance on the spectrophotometer, and the absorbance data results were compared with quercetin (Sigma Aldrich) as a standard (Mohammed & Abdullah., 2022). Total flavonoid levels are expressed in QE (Quercetin Equivalent), which is the microgram equivalent amount of quercetin in 1 gram sample (µg QE/g).

Total Phenol Content (TPC)

TPC testing was carried out using the Folin-Ciocalteu method (Shao et al., 2014). A sample with a quantity of 400 µL was added to 400 µL of 10% Folin-Ciocalteu reagent (Merck, Germany) and incubated at room temperature for 5 minutes. A total of 300 µL of 75 g/L Na_2CO_3 (Merck, Germany) was added at the end of the incubation time and incubated for 1 hour. Absorbance was observed with a UV-Vis spectrophotometer at a wavelength of 760 nm, using gallic acid (Sigma Aldrich, Germany) as a standard. The TPC of each sample was determined from the generated standard curve prepared using gallic acid (GA) ranging from 1 to 50 mg/L solutions of GA in water. The total concentration of phenol is expressed in GA, i.e. the microgram equivalent amount of GA in 1 gram sample (µg GAE/g).

RESULTS AND DISCUSSIONS

Characterization of T-SAG

The characterization of Transfersome Single Aged Garlic (T-SAG) prepared with different surfactants and solvents revealed distinct physicochemical properties. The polydispersity index (PDI), z-average, and zeta potential were assessed to evaluate the size distribution, particle size, and surface charge of the T-SAG, respectively (**Table 1**). Transfersomes are phospholipid vesicles employed as carriers for transdermal drug delivery. Formulations typically include phospholipids as vesicle-forming agents, surfactants for flexibility, absolute ethanol, chloroform, or methanol as solvents, and buffer agents for wetting (Chaurasiya et al., 2019; Opatha et al., 2020).

The characterization data presented in **Table 1** provide insights into the physicochemical properties of SAG-Transfersomes formulated with different surfactants and solvents. The PDI values, all below 0.5, indicate a relatively uniform size distribution for all the simulated transfersomes (Chaerunisaa et al., 2023). The lowest PDI value observed in the T2-EA formulation suggests a more homogeneous particle size distribution, which could potentially improve the stability and bioavailability of the encapsulated SAG. The z-average measurements reveal that the particle size of the SAG-loaded transfersomes varied significantly depending on the surfactant and solvent combination (Jiang et al., 2018). The T2-EA formulation, using Span-60 and absolute ethanol, resulted in the largest particle size. This could be attributed to the less hydrophilic nature of Span-60, which might lead to the formation of larger aggregates or micelles. In contrast, the T1-EA formulation, using Tween-80 and absolute ethanol, exhibited the smallest particle size, potentially due to the better compatibility between Tween-80 and the phospholipids, resulting in more compact and smaller transfersomes. An ideal transfersome size is below 200 nm, as vesicle size is a critical factor influencing drug encapsulation efficiency during transfersome manufacturing (Bnyan et al., 2019; Opatha et al., 2020).

The zeta potential values provide information about the surface charge of the transfersomes (Surini & Joshita Djajadisastra, 2018). A more negative zeta potential can contribute to the stability of the formulation by minimizing electrostatic repulsion between particles (Surini & Djajadisastra, 2018). The T2-EA formulation exhibited the lowest zeta potential, suggesting a potentially higher stability compared to the other formulations. On the other hand, the T2-CM formulation, using Span-60 and a chloroform-methanol mixture, displayed the highest zeta potential, which might indicate a greater tendency for aggregation. A zeta potential below -30 mV or above +30 mV is generally considered stable, preventing particle aggregation and flocculation (Zhang et al., 2015). Negative zeta potentials are favorable for stability and enhance transfersome penetration (Khan et al., 2022; Nangare et al., 2021).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Activity

The results presented in **Figure 1** demonstrate the successful preservation of antioxidant activity in SAG extract through the transfersome manufacturing process. The observed differences in antioxidant activity between transfersomes formulated with different solvents highlight the impact of the solvent on the stability and bioactivity of the encapsulated SAG. Transfersomes prepared with a chloroform-methanol solvent mixture consistently exhibited higher antioxidant activity compared to those using absolute ethanol. This could be attributed to the superior solvent properties of the chloroform-methanol mixture in preserving the bioactive compounds of SAG during the encapsulation process. Chloroform and methanol are known to be effective solvents for extracting and solubilizing plant-based compounds, including antioxidants (Jang et al., 2018).

Table 1. SAG-Transfersome characterization data results

No	Formulation	PDI	Z-Average (nm)	Zeta Potensial (mV)
1	T1-CM	0.399 ± 0.038	84.333 ± 1.762	-14.133 ± 0.839
2	T2-CM	0.372 ± 0.022	174.167 ± 3.166	-16.200 ± 0.361
3	T1-EA	0.382 ± 0.044	96.150 ± 3.889	-12.000 ± 1.058
4	T2-EA	0.409 ± 0.023	366.950 ± 48.578	-25.667 ± 0.666

Note: CM: chloroform methanol 2:1. EA: absolute ethanol. T1: formula phospolipid and tween-80; T2: formula phospolipid and span-60. PDI : polydispersity index

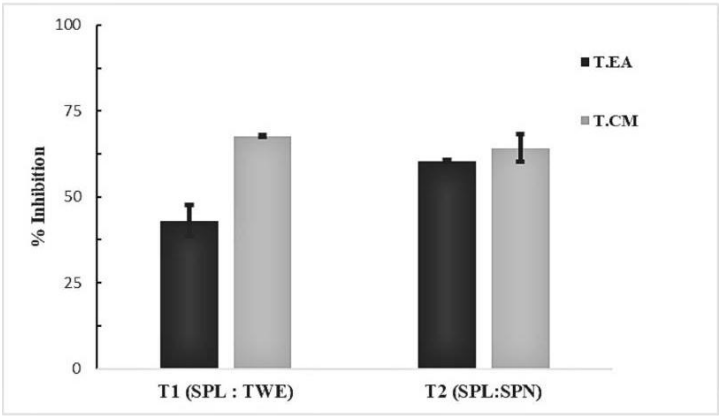

Figure 1. Antioxidant activity of transfersomes. CM: chloroform methanol. EA: absolute ethanol. T1: formula phospolipid (SPL) and tween-80 (TWE); T2: formula phospolipid (SPL) and span-60 (SPN).

The T1-EA formulation, using Tween-80 and absolute ethanol, displayed the lowest antioxidant activity. This might be due to the less efficient encapsulation of SAG in absolute ethanol, leading to a loss of bioactive compounds during the formulation process. Additionally, the less polar nature of absolute ethanol compared to the chloroform-methanol mixture might not be as conducive to maintaining the stability of the encapsulated antioxidants (Januarti et al., 2019; Li et al., 2021). Interestingly, when comparing ethanol-based transfersomes, T2-CM (using Span-60 and chloroform-methanol) exhibited slightly higher antioxidant activity than T2-EA (using Span-60 and absolute ethanol). This suggests that the choice of surfactant also plays a role in influencing the antioxidant activity of the transfersomes. The specific mechanisms underlying these differences require further investigation.

Phenolic and flavonoid compounds possess antioxidant properties, acting as free radical scavengers. Their chemical properties, including hydrogen atom donation, metal chelation, and biological activity, contribute to their antioxidant effects (Elosta et al., 2017; Mohammed & Abdullah, 2022). Previous studies reported no significant impact of encapsulation on resveratrol's antioxidant activity in transfersomes (Wu et al., 2019). Additionally, tetrahydrocurcumin's antioxidant activity was not hindered by its encapsulation or incorporation into vesicular cream (Kanshide et al., 2022). Aged garlic extract itself exhibits antioxidant activity in various solvents, including distilled water, ethanol, and chloroform (Jang et al., 2018). SAG has been shown to contain higher levels of phenols, flavonoids, and sulfur compounds (e.g., S-ally-(L)-cysteine, disulfide) compared to fresh garlic. The abundance of phenolic and flavonoid compounds correlates positively with DPPH radical scavenging activity, attributed to their ability to donate hydrogen and electrons from the hydroxyl group (Eun et al., 2014; Jang et al., 2018; Lu et al., 2017).

Total Flavonoid Content (TFC)

The data presented in Table 2 reveal variations in total flavonoid content (TFC) among the different black garlic Transfersome formulations. T2 formulations, utilizing Span-60 as the surfactant, consistently exhibited higher TFC values compared to T1 formulations, employing Tween-80. This suggests that Span-60 might be more effective in encapsulating and protecting flavonoids during the transfersome formation process. The solvent type also influenced TFC. Both T1 and T2 formulations using chloroform-methanol (CM) solvent demonstrated slightly higher TFC values than those using absolute ethanol (EA). This could be attributed to the superior solvent properties of chloroform-methanol in extracting and solubilizing flavonoids from the single aged garlic extract.

Flavonoids are the largest group of phenolic compounds that have properties as antioxidants (Hanin & Pratiwi, 2017; Safe et al., 2021). As in the total phenolic content test, the increased antioxidant activity of SAG is due to an increase in the bioactivity of flavonoid compounds, polyphenols, and the formation of SAC compounds (Sasmitaloka et al., 2022; Sembiring & Iskandar, 2019). While organosulfur compounds, such as S-allyl cysteine (SAC) and diallyl disulfide, are indeed the characteristic active compounds in garlic, our study also considers flavonoid and phenolic compounds as important bioactive components in single-aged garlic (SAG) (Dewi, 2018). Notably, black garlic, including SAG, contains polyphenolic compounds such as quercetin, kaempferol, catechin, and gallic acid, which contribute significantly to its antioxidant properties (Ryu & Kang, 2017). These compounds enhance the bioactivity of SAG and influence its pharmacological potential. Since flavonoids and phenolic compounds exhibit strong antioxidant activity, they are crucial parameters for evaluating the effectiveness of transfersomes in delivering these bioactive components (Nguyen et al., 2021; Ryu & Kang, 2017). Total flavonoid content is more identified in transfersomes with methanol and chloroform solvents than absolute ethanol solvents. Solvents like methanol and chloroform are crucial for solubilizing lipids and forming stable transfersome structures (Jiang et al., 2018; Nayak & Tippavajhala, 2021). The interaction between these solvents and lipids significantly influences the size, morphology, and cargo capacity of the vesicles, as well as the total encapsulated flavonoids. Soy lecithin, a common phospholipid used in lipid-based vesicular drug delivery systems, typically constitutes 1-4% w/w of the formulation (Chaurasiya et al., 2019; Leonyza & Surini, 2019). Soy lecithin is readily available and relatively inexpensive.

Table 2. Total flavonoid content of transfersome SAG extract

No	Formulation	Total Flavonoid Content (µg QE/g)
1	T1-EA	38.38 ± 0.68
2	T2-EA	62.55 ± 0.34
3	T1-CM	40.78 ± 0.48
4	T2-CM	66.61 ± 0.18

Note: CM: chloroform methanol 2:1. EA: absolute ethanol. T1: formula phospolipid and tween-80; T2: formula phospolipid and span-60. PDI : polydispersity index

Table 3. Total phenolic content of transfersome SAG extract

No	Formulation	Total Phenolic Content (µg GAE/g)
1	T1-EA	50.18 ± 1.73
2	T2-EA	43.83 ± 1.36
3	T1-CM	51.80 ± 2.76
4	T2-CM	49.39 ± 0.53

Note: CM: chloroform methanol 2:1. EA: absolute ethanol. T1: formula phospolipid and tween-80; T2: formula phospolipid and span-60. PDI : polydispersity index

Tween-80 and Span-60, used as penetration enhancers, are incorporated at 5-10%, while ethanol is used at 20-40%. High ethanol concentrations can tighten the lipid membrane, enhancing stability and creating a softer structure, which improves drug distribution in the stratum corneum (Leonyza & Surini, 2019; Wongrakpanich et al., 2022a). Additionally, Tween-80 and Span-60 enhance the flexibility of the lecithin vesicle's lipid bilayer, allowing transfersomes to spontaneously pass through pores smaller than their size (Khan et al., 2022; Leonyza & Surini, 2019).

Total Phenol Content (TPC)

Total phenolic content in SAG-loaded transfersomes was determined using gallic acid as a standard, due to its natural phenolic nature and stability. **Table 3** summarizes the absorbance values and total phenolic content of the T-SAG transfersomes. The data obtained from this study revealed significant variations TPC among different formulations of single aged garlic extract transfersomes. These variations were primarily attributed to differences in the types of solvents and surfactants employed in transfersome preparation.

Transfersomes formulated with CM solvents consistently exhibited higher TPC values compared to those using EA. This suggests that the solvent's polarity and its ability to dissolve phenolic compounds significantly influence encapsulation efficiency (Jahanfar et al., 2021; Vo et al., 2019). The chloroform-methanol mixture, due to the presence of chloroform as a non-polar component, effectively dissolves non-polar phenolic compounds in single aged garlic extract, facilitating their incorporation into the transfersome structure. At the same time, the presence of methanol increases the solvent system's overall polarity, improving its ability to dissolve amphiphilic components such as phospholipids and surfactants. This dual nature of the solvent system enhances the efficiency of transfersome formation by optimizing the solubility of both hydrophobic and hydrophilic compounds (Fitrya et al., 2020; Lu et al., 2017; Singh & Gaikwad, 2021). Solvents like methanol and chloroform are crucial for solubilizing lipids and forming stable transfersome structures. The interaction between these solvents and lipids significantly influences the vesicles's size, morphology, and cargo capacity and the total encapsulated phenols (Natsheh & Touitou, 2020). Soy lecithin, a commonly used phospholipid in lipid-based vesicular drug delivery systems, typically constitutes 1- 4% w/w of the formulation (Chaurasiya et al., 2019). It is readily available and relatively inexpensive. Methanol also aids in phospholipid hydration, a key component of the transfersome membrane (Abdel-Gawad et al., 2018; Mohammed & Abdullah, 2022). The interaction between methanol, phospholipids, and SAG compounds during the preparation process influences vesicle size, distribution, and encapsulation efficiency. In addition to methanol, chloroform is another solvent used in transfersome preparation. The combination of chloroform and methanol is particularly effective for dissolving Tween-80 or Span-60 surfactants and efficiently absorbing SAG extract. Chloroform's high solubility in alcohol and ether makes it a suitable solvent for use with methanol (Natsheh & Touitou, 2020).

Surfactants also played a crucial role in TPC. Tween-80, being more hydrophilic than Span-60, tended to produce formulations with slightly higher TPC values. This indicates Tween-80's superior ability to stabilize emulsions and protect phenolic compounds from degradation during the transfersome manufacturing process. The interaction between solvents, surfactants, and phenolic compounds is a complex factor influencing TPC in transfersomes (Jiang et al., 2018; Leonyza & Surini, 2019). Solvents affect the solubility of phenolic compounds, while surfactants influence micelle formation and emulsion stability (Elosta et al., 2017; Lu et al., 2017). The combination of chloroform-methanol solvent and Tween-80 surfactant proved particularly effective in maintaining TPC, likely due to its ability to form a stable transfersome structure and efficiently encapsulate phenolic compounds (Khan et al., 2022). These findings have significant implications for the development of transfersome-based formulations for bioactive compound delivery. The careful selection of solvents and surfactants can substantially impact the encapsulation efficiency and stability of active compounds within the formulation.

The combination of methanol and chloroform enhances the solvent's polarity, increasing its ability to dissolve phospholipids and surfactants like Tween-80 or Span-60, resulting in a homogeneous mixture within the transfersome formulation (Islam et al., 2021; Wongrakpanich et al., 2022b). Transfersomes formulated with organic solvents like absolute ethanol,

methanol, and chloroform exhibit promising characteristics and can effectively protect the antioxidant compounds, flavonoids, and phenols present in SAG extracts. These solvents, known for their ability to dissolve polar and nonpolar compounds, facilitate the formation of stable, well-characterized transfersomes. The combination of these solvents can enhance the encapsulation efficiency of bioactive compounds, protecting them from degradation and ensuring their controlled release. The resulting transfersomes often demonstrate desirable properties, such as small particle size, uniform distribution, and high stability, making them suitable for various applications, including drug delivery and nutraceutical formulations.

CONCLUSIONS

Transfersomes composed of phospholipids and Tween-80 were found to be the most effective formulation based on solvent type. Among the solvents tested, ethanol was superior in preserving antioxidant activity compared to chloroform and methanol. Furthermore, the SAG transfersomes formulated using a chloroform-methanol solvent mixture exhibited superior phytochemical characteristics and a higher content of phenolic and flavonoid compounds compared to those formulated with absolute ethanol. This suggests that the chloroform-methanol mixture provided better protection and retention of these bioactive compounds within the transfersome structure. To further support these findings, additional research is necessary to investigate encapsulation efficiency, drug release, and other in vitro parameters.

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REFERENCES

Abdel-Gawad, M., Abdel-Aziz, M., El-Sayed, M., El-Wakil, E., & Abdel-Lateef, E. (2018). in vitro antioxidant, total phenolic and flavonoid contents of six allium species growing in Egypt. *Journal of Microbiology, Biotechnology and Food Sciences*, *8*(2), 343–346.

Bnyan, R., Khan, I., Ehtezazi, T., Saleem, I., Gordon, S., & Neill, F. O. (2019). *Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic*. https://doi.org/10.1111/jphp.13149

Capasso, A. (2013). Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Molecules*, *18*(1), 690–700. https://doi.org/10.3390/molecules18010690

Chaerunisaa, Z., Abdassah, A., & Darajat, M. Z. (2023). Transfersome as topical drug delivery: formulation and characterization. *Galenika Journal of Pharmacy) (e-Journal)*, *9*(1), 41–54. https://doi.org/10.22487/j24428744.2023.v9.i1.16030

Chang, W. T., Lim, J. L., & Hsu, C. L. (2018). Inhibitory effect of methanolic extract of black garlic on adipogenesis in 3T3-L1 adipocytes. 中山醫學雜誌, 113–121.

Chaurasiya, P., Ganju, E., Upmanyu, N., Ray, S. K., & Jain, P. (2019). Transfersomes: a novel technique for transdermal drug delivery. *Journal of Drug Delivery and Therapeutics*, *9*(1), 279–285. https://doi.org/10.22270/jddt.v9i1.2198

Choi, I. S., Cha, H. S., & Lee, Y. S. (2014). *Physicochemical and Antioxidant properties of black garlic*. 16811–16823. https://doi.org/10.3390/molecules191016811

Dewi, N. N. A. (2018). Nutrition content and antioxidant activity of black garlic. *International Journal of Health Sciences (IJHS)*, *2*(1). https://doi.org/10.29332/ijhs.v2n1.86

Elosta, A., Slevin, M., Rahman, K., & Ahmed, N. (2017). Aged garlic has more potent antiglycation and antioxidant properties compared to fresh garlic extract in vitro. *Scientific Reports*, *7*(June 2016), 1–9. https://doi.org/10.1038/srep39613

Eun, S., Yong, S., Duk, Y., Ha, S., & Jin, H. (2014). LWT - Food science and technology changes in S -allyl cysteine contents and physicochemical properties of black garlic during heat treatment. *LWT - Food Science and Technology*, *55*(1), 397–402. https://doi.org/10.1016/j.lwt.2013.05.006

Fitrya, F., Fithri, N. A., Winda, M., & Muharni, M. (2020). Ethanol extract of *Parkia speciosa* Hassk. loaded transfersome: Characterization and optimization [Transfersoma cargado con extracto etanólico de Parkia speciosa Hassk.: caracterización y optimización]. *Journal of Pharmacy & Pharmacognosy Research*, *8*(3), 167–176. http://jppres.com/jppreshttp://jppres.com/

Hanin, N. N. F., & Pratiwi, R. (2017). Kandungan fenolik, flavonoid dan aktivitas antioksidan ekstrak daun paku laut (*Acrostichum aureum* L.) fertil dan steril di Kawasan Mangrove Kulon Progo, Yogyakarta (Phenolic, flavonoid content and antioxidant activity of fertile and sterile sea fern (*Acrostichum aureum* L.) leaf extract in Kulon Progo Mangrove Area, Yogyakarta). *Journal of Tropical Biodiversity and Biotechnology*, *2*(2), 51. https://doi.org/10.22146/jtbb.29819

Ilmawati, R. R., Amin, A. Z., & Amin, M. (2017). Alliin

as a natural bioactive from single bulb garlic (*Allium sativum)* for nitric oxide (NO) increasing in atherosclerotic process based on insilico Screening. *Biology, Medicine, & Natural Product Chemistry*, *6*(2), 59. https://doi.org/10.14421/biomedich.2017.62.59-62

Islam, N., Irfan, M., Zahoor, A. F., Iqbal, M. S., Syed, H. K., Khan, I. U., Rasul, A., Khan, S. U. D., Alqahtani, A. M., Ikram, M., Qayyum, M. A., Khames, A., Inam, S., & Abourehab, M. A. S. (2021). Improved bioavailability of ebastine through development of transfersomal oral films. *Pharmaceutics*, *13*(8), 1–20. https://doi.org/10.3390/pharmaceutics13081315

Jahanfar, S., Gahavami, M., Khosravi-Darani, K., Jahadi, M., & Mozafari, M. R. (2021). Entrapment of rosemary extract by liposomes formulated by Mozafari method: physicochemical characterization and optimization. *Heliyon*, *7*(12), e08632. https://doi.org/10.1016/j.heliyon.2021.e08632

Jang, H. J., Lee, H. J., Yoon, D. K., Ji, D. S., Kim, J. H., & Lee, C. H. (2018). Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. *Food Science and Biotechnology*, *27*(1), 219–225. https://doi.org/10.1007/s10068-017-0246-4

Jang, J. Y., Lee, M. J., You, B. R., Jin, J. S., Lee, S. H., Yun, Y. R., & Kim, H. J. (2017). Allium hookeri root extract exerts anti-inflammatory effects by nuclear factor-κB down-regulation in lipopolysaccharide-induced RAW264.7 cells. *BMC Complementary and Alternative Medicine*, *17*(1), 1–10. https://doi.org/10.1186/s12906-017-1633-3

Januarti, I. B., Taufiq, H., & Sulistyaningsih. (2019). The Correlation of total flavonoid and total phenolic. *Jurnal Farmasi Sains Dan Komunitas*, *16*(2), 96–103.

Jiang, T., Wang, T., Li, T., Ma, Y., Shen, S., He, B., & Mo, R. (2018). Enhanced transdermal drug delivery by transfersome-embedded oligopeptide hydrogel for topical chemotherapy of melanoma. *ACS Nano*, *12*(10), 9693–9701. https://doi.org/10.1021/acsnano.8b03800

Kanshide, A., Rao, M., Nagesh, P., Arzoo, C., Vijay, J., & Manohar, K. (2022). Formulation , optimization , and antioxidant evaluation of tetrahydrocurcumin - loaded ultradeformable nanovesicular cream. *Journal of Pharmaceutical Innovation*. https://doi.org/10.1007/s12247-022-09696-0

Khan, M. I., Yaqoob, S., Madni, A., Akhtar, M. F., Sohail, M. F., Saleem, A., Tahir, N., Khan, K. ur R., & Qureshi, O. S. (2022). Development and in vitro / ex vivo evaluation of lecithin-based deformable transfersomes and transfersome-based gels for combined dermal delivery of meloxicam and dexamethasone. *BioMed Research International*, *2022*. https://doi.org/10.1155/2022/8170318

Krishna, S. A. (2023). Transferosomes-a novel carrier for drug delivery. *Dermatology and Dermatitis*, *8*(5), 01–05. https://doi.org/10. 31579/2578-8949/132

Lawson, L. D., & Hunsaker, S. M. (2018). Allicin bioavailability and bioequivalence from garlic supplements and garlic foods. In *Nutrients, 10*(7). https://doi.org/10.3390/nu10070812

Lee, J. Y., & Gao, Y. (2012). Review of the application of garlic, *Allium sativum*, in aquaculture. *Journal of the World Aquaculture Society*, *43*(4), 447–458. https://doi.org/10.1111/j.1749-7345.2012.00581.x

Leonyza, A., & Surini, S. (2019). Optimization of sodium deoxycholate-based transfersomes for percutaneous delivery of peptides and proteins. *International Journal of Applied Pharmaceutics*, *11*(5), 329–332. https://doi.org/10.22159/ijap.2019v11i5.33615

Li, N., Chen, K., Dong, H., Yang, J., Yoshizawa, M., Kagami, H., & Li, X. (2021). Alliin inhibits adipocyte differentiation by downregulating Akt expression: Implications for metabolic disease. *Experimental and Therapeutic Medicine*, *21*(6), 1–8. https://doi.org/10.3892/etm.2021.9995

Lu, X., Li, N., Qiao, X., Qiu, Z., & Liu, P. (2017). Composition analysis and antioxidant properties of black garlic extract. *Journal of Food and Drug Analysis*, *25*(2), 340–349. https://doi.org/10.1016/j.jfda.2016.05.011

Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2021). Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery*, *20*(2), 101–124. https://doi.org/10.1038/s41573-020-0090-8

Mohammed, H. H., & Abdullah, F. O. (2022). Microwave-assisted extraction and phytochemical profile of nonea pulmonarioides and its antifungal, antibacterial, and antioxidant activities. *Journal of Food Quality*, *2022*. https://doi.org/10.1155/2022/5135880

Nangare, S., Bhatane, D., Mali, R., & Shitole, M. (2021). Development of a novel freeze-dried mulberry leaf extract-based transfersome gel. *Turkish Journal of Pharmaceutical Sciences*, *18*(1), 44–55. https://doi.org/10.4274/tjps.galenos.2019.98624

Natsheh, H., & Touitou, E. (2020). Phospholipid vesicles for dermal/transdermal and nasal administration of active molecules: The effect of surfactants and alcohols on the fluidity of their lipid bilayers and penetration enhancement properties. In *Molecules* (Vol. 25, Issue 13). MDPI AG. https://doi.org/10.3390/molecules

25132959

Nayak, D., & Tippavajhala, V. K. (2021). A comprehensive review on preparation, evaluation and applications of deformable liposomes. In *Iranian Journal of Pharmaceutical Research* (Vol. 20, Issue 1, pp. 186–205). Iranian Journal of Pharmaceutical Research. https://doi.org/10.22037/ijpr.2020.112878.13997

Nguyen, T. T. H., Jung, J.-H., Kim, M.-K., Lim, S., Choi, J.-M., Chung, B., Kim, D.-W., & Kim, D. (2021). The Inhibitory effects of plant derivate polyphenols on the main protease of SARS coronavirus 2 and their structure-activity relationship. *Molecules (Basel, Switzerland)*, *26*(7). https://doi.org/10.3390/molecules26071924

Okonogi, S., Phumat, P., Khongkhunthian, S., Chaijareenont, P., Rades, T., & Müllertz, A. (2021). Development of self-nanoemulsifying drug delivery systems containing 4-allylpyrocatechol for treatment of oral infections caused by *Candida albicans*. *Pharmaceutics*, *13*(2), 1–16. https://doi.org/10.3390/pharmaceutics13020167

Omar, M. M., Hasan, O. A., & El Sisi, A. M. (2019). Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: A promising approach for enhancement of skin permeation. *International Journal of Nanomedicine*, *14*, 1551–1562. https://doi.org/10.2147/IJN.S201356

Opatha, S. A. T., Titapiwatanakun, V., & Chutoprapat, R. (2020). Transfersomes: A promising nanoencapsulation technique for transdermal drug delivery. In *Pharmaceutics. 12*(9), 855.

Qadariah, N., Lestari, S. R., & Rohman, F. (2020). Single bulb garlic (*Allium sativum*) extract improve sperm quality in hyperlipidemia male mice model. *Jurnal Kedokteran Hewan - Indonesian Journal of Veterinary Sciences*, *14*(1), 7–11. https://doi.org/10.21157/j.ked.hewan.v14i1.13562

Rasheed, M. S., Ansari, S. F., & Shahzadi, I. (2022). Formulation, characterization of glucosamine loaded transfersomes and in vivo evaluation using papain induced arthritis model. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-23103-1

Ryu, J. H., & Kang, D. (2017). Physicochemical properties, biological activity, health benefits, and general limitations of aged black garlic: A Review. *Molecules (Basel, Switzerland)*, *22*(6). https://doi.org/10.3390/molecules22060919

Sachan, R., Parashar, T., Singh, V., Singh, G., Tyagi, S., Patel, C., & Gupta, A. (2013). Drug carrier transfersomes: A novel tool for transdermal drug delivery system. *International Journal of Research and Development in Pharmacy and Life Sciences*, *2*(2), 309-316.

Safe, S., Jayaraman, A., Chapkin, R. S., Howard, M., Mohankumar, K., & Shrestha, R. (2021). Flavonoids: structure–function and mechanisms of action and opportunities for drug development. *Toxicological Research*, *37*(2), 147–162. https://doi.org/10.1007/s43188-020-00080-z

Sasmitaloka, K. S., Widayanti, S. M., Mulyawanti, I., & Iriani, E. S. (2022). Physicochemical and antioxidant characteristics of black garlic from indigenous Indonesian garlic. *IOP Conference Series: Earth and Environmental Science*, *1041*(1). https://doi.org/10.1088/1755-1315/1041/1/012004

Sembiring, N. B., & Iskandar, Y. (2019). A Review of component and pharmacology activities of black garlic. *Majalah Obat Tradisional*, *24*(3), 178. https://doi.org/10.22146/mot.45277

Shao, Y., Xu, F., Sun, X., Bao, J., & Beta, T. (2014). Identification and quanti fi cation of phenolic acids and anthocyanins as antioxidants in bran , embryo and endosperm of white , red and black rice kernels (*Oryza sativa* L .). *Journal of Cereal Science*, *59*(2), 211–218. https://doi.org/10.1016/j.jcs.2014.01.004

Sharma, V., Yusuf, M., & Pathak, K. (2014). Nanovesicles for transdermal delivery of felodipine: Development, characterization, and pharmacokinetics. *International Journal of Pharmaceutical Investigation*, *4*(3), 119. https://doi.org/10.4103/2230-973x.138342

Singh, V., & Gaikwad, M. (2021). *Development and evaluation of transferosomal gel using cephalexin*. https://doi.org/10.21203/rs.3.rs-940323/v1

Surini, S., & Djajadisastra, S. (2018). Formulation and in vitro penetration study of transfersomes gel containing gotu kola leaves extract (*Centella asiatica* L. Urban). *Journal of Young Pharmacists*, *10*(1), 27–31. https://doi.org/10.5530/jyp.2018.10.8

Suryawanshi, S., Shailendra Suryawanshi Sanjay, C., & Kabbinavar, S. (2018). Phytochemicals and potential biological activities of *Allium sativum* Linn. *~ 662 ~ Journal of Pharmacognosy and Phytochemistry*, *7*(6), 662–670.

Tran, G. B., Dam, S. M., & Le, N. T. T. (2018). Amelioration of single clove black garlic aqueous extract on dyslipidemia and hepatitis in chronic carbon tetrachloride intoxicated Swiss Albino mice. *International Journal of Hepatology*, *2018*. https://doi.org/10.1155/2018/9383950

Vo, Q. V., Nam, P. C., Thong, N. M., Trung, N. T., Phan, C. T. D., & Mechler, A. (2019). Antioxidant motifs in flavonoids: O−H versus C−H bond dissociation. *ACS Omega*, *4*(5), 8935–8942.

https://doi.org/10.1021/acsomega.9b00677

Wongrakpanich, A., Leanpolchareanchai, J., Morakul, B., Parichatikanond, W., & Teeranachaideekul, V. (2022a). Phyllanthus emblica extract-loaded transfersomes for hair follicle targeting: phytoconstituents, characterization, and hair growth promotion. *Journal of Oleo Science*, *71*(7), 1085–1096. https://doi.org/10.5650/jos.ess21425

Wu, P. S., Li, Y. S., Kuo, Y. C., Tsai, S. J. J., & Lin, C. C. (2019). Preparation and evaluation of novel transfersomes combined with the natural antioxidant resveratrol. *Molecules*, *24*(3), 1–12. https://doi.org/10.3390/molecules24030600

Zhang, L., Zhang, L., Zhang, M., Pang, Y., Li, Z., Zhao, A., & Feng, J. (2015). Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug Delivery*, *22*(4), 475–486. https://doi.org/10.3109/10717544.2013.861659