Formulation and Evaluation of Hyaluronate-Coated Novasomes Loaded with Beta vulgaris subsp. Cicla Arial Parts Extract (Swiss Chard) Cultivated in Egypt Along with Phytochemical Investigation

Doaa A. H. Deabes
National Research Centre
Eman A.W. El-abd
National Research Centre
Alaa M. Saleh
alaasaleh011@gmail.com
National Research Centre
Eman S. Shalaby
National Research Centre

Article

Keywords: Antimicrobial, Antioxidant, GC/MS, LC/MS/MS, Novosomes

Posted Date: January 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8535761/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on June 16th, 2026. See the published version at https://doi.org/10.1038/s41598-026-56921-8.

Formulation and Evaluation of Hyaluronate-Coated Novasomes Loaded with *Beta vulgaris subsp. Cicla* Arial Parts Extract (Swiss Chard) Cultivated in Egypt Along with Phytochemical Investigation

Doaa A. H. Deabes[1], Eman A.W. El-abd[1], Alaa M. Saleh[2], Eman S. Shalaby[3]

[1] Pharmacognosy Department, National Research Centre, 33El Buhouth St., Dokki, Giza, P.O.12622 (ID: 60014618), Egypt.
[2] Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Research Institute, National Research Centre, 33El Buhouth St., Dokki, Giza, P.O.12622 (ID: 60014618), Egypt.
[3] Pharmaceutical technology department, National Research Centre, 33El Buhouth St., Dokki, Giza, P.O.12622 (ID: 60014618), Egypt.

Corresponding author:
Alaa M. Saleh
Email: alaasaleh011@gmail.com
https://orcid.org/0000-0002-9076-3102

Abstract:
Arial parts extracts of *Beta vulgaris subsp. Cicla* (BV) along with their novasomes coated with hyaluronate formulations (BV1 and BV2) were investigated for their antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231 and their scavenging potential against (DPPH) radical. The selected formulation BV1 was tested for encapsulation efficiency, particle size zeta potential, FTIR and TEM., Fifteen chromatographic peaks were identified by GC/MS analysis represented (89.64%) from BV dichloromethane fraction, the most abundant compound was oleic acid (30.52%), followed by Palmitic acid (21.92%) and Stearic acid (10.50%) triterpenes and sterol also identified. LC/MS/MS of total ethanol extract results the identification of 33 compounds including flavonoids, phenolic acids and their derivatives which represented the major compounds also coumarin, fatty acid and amino acid were identified. In addition to isolation of four compounds Oleanolic acid and campsterol from dichloromethane fraction while isovitexin and Apigenin-O- hexoside from ethanol extract. The crude extract showed antimicrobial activity against *C.albicans* only while novasomes formulations BV1 was active against both bacteria and yeast. As for BV2, it was active against bacteria only. Regarding antioxidant activity, crude extract together with both formulas showed ability to scavenge DPPH free radical.

Keywords: Antimicrobial • Antioxidant• GC/MS • LC/MS/MS • Novosomes

Abbreviations: BV, *Beta vulgaris subsp. Cicla;* BV1, Formula 1; BV2, Formula 2; FTIR, Fourier transfer infra-red; TEM, transmission electron microscope; GC/MS, Gas chromatography- mass spectroscopy; LC/MS/MS, Liquid chromatography- mass spectroscopy; DPPH, 1,1-diphenyl-2-picrylhydrazil.

Introduction

Microbial infections occur when pathogenic microorganisms such as bacteria, viruses, and fungi enter the body and multiply, leading to illness. These infections can cause a range of symptoms, including fever, pain, and swelling, and their severity varies from mild to life-threatening.

The development of bacterial resistance to drugs and the side effects associated with the use of chemical therapeutic agents made it essential for scientists to search for natural alternatives for such hazardous chemicals. Plants secondary metabolites are being thoroughly investigated for their bioactivities. Oxidative stress as a result of pollution and cellular metabolism occurring in living cells due to accumulation of reactive oxygen species such as H_2O_2 and $OH^{\cdot}$ may lead to chronic diseases including cancer, atherosclerosis and Alzheimer in addition to autoimmune diseases. Thus, the administration of antioxidant agents especially those driven from natural sources is crucial to avoid such drawbacks**[1].**

One of the significant botanical families is the *Chenopodiaceae*, or goosefoot, family. Widely found in the steppe, desert, and saline-alkaline regions of southern Africa, Central Asia and South America as well as along the Mediterranean and Red sea coasts, it is one of the most interesting flowering plants in terms of taxonomy and diagnostics **[2,3]**.

One member of *Chenopodiaceae* family, *Beta vulgaris* L. Subsp*cicla*, is an edible plant from Egypt that is referred to as Swiss chard in English.

Although many Mediterranean populations are annuals, it can flourish as a biennial or a perennial under the right environ ments.It lacks a swollen root and has long, wedgeshaped leaves arranged in a rosette **[3].** *Beta vulgaris* subsp. *cicla* is well known for its high bioactive compounds and minerals content. Thus, it is believed to have potential as antioxidant and antimicrobial in addition to other biological activities **[4,5]**. It is one of the healthiest vegetables and considered a good source of fiber, betalains, flavonoid, saponins, b-carotene, vitamin K, minerals like potassium and magnesium. Numerous investigations have demonstrated the antioxidant properties of several Swiss chard extracts and their constituents, The flavonoids, 2'',2'''-di-O-α-rhamnopyranosyl-vicenin II, a di-C-glycosyl flavone, and herbacetin 3-O-β-xylopyranosyl- (1'''→2'')-O-β-glucopyranoside, were isolated from the leaves of *Beta vulgaris* subspecies cicla L. subsp. flavescens, additional to flavonoids, 6-C-glucosyl isoscutellarein, vitexin-(1'''→2'')-O-β-xylopyranosyl, vitexin-(1'''→2'')-O-α-rhamnopyranosyl and vitexin also the hepatoprotective and hypolipidemic activities of aqueous methanolic were evaluate **[3]** in addition to their antimitotic and antiproliferative effects on human breast cancer MCF-7 cells and human colon cancer, as well as their hypoglycemic **[6,7]** Betavulgarin isolated from *Beta vulgaris* sduppresses breast cancer stem cells through Stat3 signaling **[8]**. Chard may provide a natural source of antioxidant and antiacetylcholinesterase , immnomodulatory hepatoprotective, hypoglycemic , lipid-lowering effects activities and proline content **[9,10]**. Assumed to have improved liposomal or niosomal structure, novasomes are usually composed of a combination of cholesterol, free fatty acid (FFA), and polyoxyethylene fatty acid monoester **[11]**. Novasomes are multi-bilayered vesicles that may transport a large amount of active substances because of their high-capacity central core in a small size range. To achieve the structural surface modification of the generated novasomes, one polymeric polysaccharide was studied. The polymer hyaluronic acid (HA) comes from nature. HA is bacteriostatic, but not bactericidal, and exhibits dose-dependent effects on different microorganisms in the planktonic phase. Concerning to possible orthopaedics applications, the analysis of different coatings on titanium surfaces has shown that HA significantly decreased *S. aureus* adherence **[12]**. It is an anionic glycosaminoglycan polymer made up of N-acetyl-D-glucosamine, D-glucuronic acid, and disaccharides. For functionalizing nanovesicular systems including liposomes, niosomes, spanlastics, and novasomes, HA offers a flexible polymer **[13]**. HA surface-modified nanovesicles can improve stealth qualities, prolong the action, prevent drug leakage, and slow drug clearance **[14,15]**. HA modified nanoparticles compete with native HA to provide effective drug transport into cells **[13]** .

Results and Discussion

I. Phytochemical investigation

GC/MS identified metabolites of dichloromethane fraction of aerial parts of *Beta vulgaris* subsp. Cicla

GC/MS analysis of dichloromethane fraction of arial parts of *Beta vulgaris* subsp. *Cicla* represented in Table (1) revealed the identification of fifteen oxygenated compounds representing (89.64%). the most abundant compounds oleic acid (30.52%) which is *a mono-unsaturated omega-9 fatty acid* followed by saturated fatty acid Palmitic acid (21.92%) that previously identified before in Swiss chard **[16].**

followed by Stearic acid (10.50%) triterpenes and sterol also identified in the plant (Ursane-3,12-diol, Cholesteryl benzoate, Stigmast-5-en-3-ol, oleate, R1-Barrigenol and Olean-12-ene-3,15,16,21,22,28-hexol)

Table (1): Identified compounds by GC/MS analysis of the dichloromethane fraction of arial parts of *Beta vulgaris* subsp. *Cicla*

No	Name	RRt	M.WT	M.F	Relative Area%
1	Palmitic acid	0.89	256	$C_{16}H_{32}O_2$	21.92
3	Elaidic acid, methyl ester	0.97	296	$C_{19}H_{36}O_2$	5.38
4	Nonanoic acid, 9-(o-propylphenyl)-, methyl ester	0.98	290	$C_{19}H_{30}O_2$	5.51
5	Methyl stearate	0.99	298	$C_{19}H_{38}O_2$	5.25
6	Oleic Acid	1	282	$C_{18}H_{34}O_2$,	30.52
8	Stearic acid	1.01	284	$C_{18}H_{36}O_2$,	10.50
9	6,9-Octadecadienoic acid, methyl ester	1.02	294	$C_{19}H_{34}O_2$	3.80
10	Oxiraneundecanoic acid, 3-pentyl-, methyl ester	1.03	312	$C_{19}H_{36}O_3$	1.85
11	Ursane-3,12-diol	1.24	444	$C_{30}H_{52}O_2$	1.61
12	Cholesteryl benzoate	1.35	490	$C_{34}H_{50}O_2$	0.66
13	Stigmast-5-en-3-ol, oleate	1.43	678	$C_{47}H_{82}O_2$	1.04
14	R1-Barrigenol	1.52	506	$C_{30}H_{50}O_6$	0.65
15	Olean-12-ene-3,15,16,21,22,28-hexol	1.62	506	$C_{30}H_{50}O_6$	0.95
Total identified compounds					89.64

RRt.: relative retention time relative to Oleic Acid M.F: molecular formula

M.wt: molecular weight

Metabolites identified by LC/MS/MS of arial parts of *Beta vulgaris subsp. Cicla*

The crude ethanol extract of ***Beta vulgaris* subsp. *Cicla* arial parts** was subjected to LC-MS-MS under the previous conditon, commonly used technique for the identification of different metabolites. Metabolites were assigned based on comparing the retention time (Rt), MS data highly accurate mass, molecular ion fragmentation pattern, the predicted formulas data in the literature and databases listed in the authorized websites; PubChem and Mass bank of North America (MoNA) together with the data base (Peakview software program). The ionization of compounds in th our study was performed in the negative ionization mode. The identified compounds and their mass fragments as well as molecular formula are listed in Table (2). Annotations of different chromatographic peaks allowed the identification of 33 compounds belonging to various metabolite classes were derived including flavonoids, phenolic acids and their derivatives which represented the major compounds also coumarin, fatty acid and amino acid were identified.

Table (2): Metabolites identified by LC/MS/MS of arial parts of *Beta vulgaris subsp. Cicla* in negative ionization mode.

No.	Identification	Rt.	Parent ion (M-H)	Product ion	M.F.	Chemical class	References
1	Dihydroferulic acid	1.31	195	177(M-H-H_2O),162(M-H-CO_2-CH_3),136 (M-H-H_2O-CH_3)	$C_{10}H_{12}O_4$	Phenolic acid	**[17]**
2	Ferulic acid	1.35	193	178(M-H-CH_3),149(M-HCOO),134(M-H-CH_3-CO_2)	$C_{10}H_{10}O_4$	Phenolic acid	**[17]**
3	Sinapaldahyde	2.28	207	192(M-H-CH_3),177(M-H-2CH_3),133	$C_{11}H_{12}O_4$	Phenolic acid	**[17]**
4	Protocatechuic acid	3.1	153	135[M-H-H_2O],109[M-H-CO_2], 112	$C_7H_6O_4$	Phenolic acid	**[18]**
5	P-Coumaric acid	4.09	163	145[M-H_2O],119[M-H-CO_2]	C_9H_8O	Phenolic acid	**[19]**
6	Vanillic acid	4.27	167	152(M-H-CH_3)123(M-H-CO_2),108(M-H-CH_3-CO_2)	$C_8H_8O_4$	Phenolic acid	**[17]**
7	*p*-Coumaric acid hexose	5.26	325	207, 163, 145, 119		Phenolic acid Derivative	**[20]**
8	Isovitexin 2"-O-rhamnoside	5.30	577	341(M-H-90-146),323(M-H-90-146-18),293	$C_{27}H_{30}O_{14}$	Flavonoid	**[17]**
9	Isovitexin (Apiginin 6-*C* glucoside)	5.49	431	413,341,311,283	$C_{21}H_{20}O_{10}$	Flavonoid *C*-glycoside	**[17]**
10	apigenin -C pentoside-C-hexoside	5.54	563	413,293,311	$C_{26}H_{28}O_{14}$	Flavonoid	**[10]**
11	Vitexin	6.28	431	341,311,283	$C_{21}H_{20}O_{10}$	Flavonoid C-glycoside	**[17]**
12	Apigenine-o-hexoside	6.28	431	269[M-H-hexoside], 227	$C_{21}H_{20}O_{10}$	Flavonoid	**[21]**

13	Quercetin-O-hexoside	6.35	463	301[M-H-GLU], 271[M-H-GLU-CH_2O], 255[M-H-GLU-CO-H_2O]	$C_{21}H_{19}O_{12}$	Flavonoid	**[22]**
14	Rutin	6.4	609	301[M-H-rutinoside],271[M-H-rutinoside - CH_2O],255[M-H-rutinoside-CO-H_2O],179,151	$C_{27}H_{30}O_{16}$	Flavonoid	**[17]**
15	Diosmetin 8-C-Glucoside-6-C-pentoside	6.54	593	473(M-H-120),429(M-H-120-CO_2), 413(M-H-120-60), 298,297	$C_{34}H_{25}O_{10}$	Flavonoid	**[17]**
16	Vitexin-2'-O-rhamnoside	6.86	577	457(M-H-120),311(M-H-120-146),293	$C_{27}H_{30}O_{14}$	Flavonoid	**[17]**
17	Kaempferol-O-rutinoside	6.9	593	285[M-H-rutinoside], 255[M-H-rutinoside-CH_2O],227[M-H-rutinoside-2CHO]	$C_{27}H_{30}O_{15}$	Flavonoid	**[17]**
18	Luteolin-8-C-β-D-glucopyranoside-7-O-rhamnoside	6.97	593	473(M-H-120),327(M-H-120-146),298	$C_{27}H_{30}O_{15}$	Flavonoid	**[23]**
19	Kaempferol 7-O-hexoside	7.16	447	285, 255, 227		Flavonoid *O*-glycoside	**[17]**
20	Luteolin-O-hexoside	7.16	447	401,285, 267, 255,	$C_{21}H_{20}O_{11}$	Flavonoid	**[17]**
21	Apigenin	8.17	269	251,225,197,149		Flavonoid	**[17]**
22	Trihydroxy octadecadienoic acid	8.35	327	309,229,211,171		Fatty acid	**[17]**
23	Epicatechin	8.51	289	245(M-H-CO_2)	$C_{15}H_{14}O_6$	Flavonoid	**[17]**
24	Quercetin	9.46	301	273,255, 151,179	$C_{15}H_{10}O_7$	Flavonoid	**[24]**

25	Dicaffeoylquinic acid methyl ester isomer	10.33	529	367, 353,193	$C_{26}H_{26}O_{12}$	Phenolic acid derivatives	**[25]**
26	Proline-betaxanthin	10.67	309	283, 195	$C_{14}H_{16}N_2O_6$	Betaxanthins derevatives	**[26]**
27	Chrysoeriol	10.71	299	284(M-H-CH_3),137	$C_{16}H_{12}O_6$	Flavonoid	**[17]**
28	Limocitrin	11.42	345	330,315,276	$C_{17}H_{14}O_8$	Flavonoid	**[17]**
29	Grevilline B	12.57	339	321,307,295,265	$C_{18}H_{11}O_7$	Catechols	**[27]**
30	Syringic acid	13.11	197	182(M-H-CH_3),179(M-H-H_2O),153(M-H-CO_2),129,113,85	$C_9H_{10}O_5$	Phenolic acid	**[17]**
31	Esculin (6,7-dihydroxy coumarin 6- glucoside)	12.75	339	321,293,177	$C_{15}H_{16}O_9$	Coumarin glycoside	**[17]**
32	Glutamine-betaxanthin	13.33	340	321, 277	$C_{14}H_{17}N_3O_7$	Amino acid	**[28]**
33	Chrysin (5,7-Dihydroxy flavone)	20.22	253	235 (M-H-CH_3),209	$C_{15}H_{10}O_4$	Flavonoid	**[17]**

Rt: retention time, M.F.: molecular formula

Isolation and identification of the major compounds from dicloromethane fraction

The dicloromethane extract was loaded repeatedly to TLC chromatography then, the collected bands were examined under UV at λ max 254 and 365 nm. The bands were further purified several times resulting in isolation of two compounds (1-2) that showed positive Salkowski's results and attained different colors with 10% sulphuric acid as spraying reagent

Compound 1: (Oleanolic acid)

White crystals soluble in chloroform (Rf= 0.54)

The 1H-NMR spectrum of Oleanolic acid shows numerous methyl groups at 0.80, 0.85, 0.90, 0.92,.98, 1.10, and 1.18, and a characteristic olefinic proton of C12–C13 double-bonde pentacyclic triterpenoid at 5.40 (1H, brs, H-12). This suggests an olea-12-ene skeleton. One methyl proton at 3.34 (1H, t, J = 8.2 Hz, 3_-H) is compatible with at least one hydroxyl group on the Oleanolic acid olean-12-ene-skeleton. On the other hand, the 13C-NMR spectrum displays signals related to an oxygenated

carbon signal at 78.88 (C-3), one tri-substituted double bond at 122.18 (C-12) and 144.45 (C-13) and one carboxyl group at 180.64 (C-28). Further, 13C-NMR signals from C-18 to C-22 at (41.68 (C-18), 46.35 (C-19), 30.52 (C-20), 32.88 (C-21) and 31.55 (C-22)) indicates that Oleanolic acid derives from the oleanyl carbocation.The MS spectra for OA obtained by LC-ESI-ITTOF, finding that the five most prominent peaks corresponded to the m/z molecular fragments 457,203,191,218,75 **[29,30].**

Compound 2: (Campesterol)

It had a melting point of 160-164 ^{0}C and was obtained in the form of a white powder. EI-MS showed characteristic peaks at an m/z of 400, with molecular ion and fragment ion m/ z values of 382,367,273 and 255 indictaing a molecular formula of $C_{28}H_{48}O$. The H- NMR spectra data was almost identical to that of campesterol in the literature, with 1H - NMR displaying dH for one olefinic methine proton (dH 5.46 H 6), one hydroxyl proton (dH 4.49 OH), six methyl protons (dH 0.76 H-18, 0.63 H-19, 0.76 H-21, 0.73 C-26, 0.79 H-27, and 0.62 C-28), ten methylene protons (dH 1.94 H-1, 1.78 H-2, 1.56 H-4, 1.10 H-7, 1.09 H-11, 1.16 H-12, 2.09 H-15, 1.86 H-16, 2.15 H-22, and 1.04 H-23), and eight methine protons (dH 3.48 H-3, 1.76 H-8, 0.95 H-9, 1.42 H-14, 1.75 H-17, 2.23 H-20, 0.85 H-24, and 1.22 H-25).

The ^{13}C NMR and DEPT revealed 28 carbon signals for six methyl carbons (dC 15.54 C-18, 12.14 C-19, 14.22 C-21, 21.23 C-26, 19.88 C-27, and 15.54 C-28), ten methylene carbons (dC 37.40 C-1, 31.79 C-2, 42.43 C-4, 32.08 C-7, 23.18 C-11, 39.88 C-12, 24.42 C-15, 26.18 C-16, 34.16 C 22, and 34.10 C-23), eight methine carbons (dC 70.01 C-3, 31.06 C-8, 49.42 C-9, 54.93 C-14, 53.10 C-17, 35.30 C-20, 38.21 C-24, and 31.86C-25), three quaternary carbons (dC 143.41 C-5, 35.66 C-10, and 43.95 C-13), and one olefinic methine carbon (dC 121.92 C-6) Compound 1 was identified as campesterol in accordant with the spectral data that stated in the literature **[31].**

Compounds isolated from ethanol extract:

Compound 3: (Isovitexin)

Yellow powder soluble in methanol, Rf. 0.73 In system (CH_2CL_2: MeOH 9:1) UV λ_{max} in methanol 334,270 which indicate flavone class, sodium methoxide 395,327,280 that revealed the presence of free 4' OH, HCl/ $ALCl_3$ 390,344,304,278 which showed that there is no ortho dihydroxy group and in NaOAC/ Boric acid 344,304,272.^{1}H NMR (400MHz, MeOH) 7.96(2H, d, J= 8.6 2',6'H), 6.92 (2H, d, J= 8.6 ,3',5'H), 6.60 (1H, s, 8-H), 6.26 (1H, s, 3-H), 5.00 (1H, d, J=9.92 H1") C-sugar, 4.84(1H, s). TOF/MS-ESI $(431)^{-}$ (M-H) ,413(M-H-H_2O),341(M-H-90),311(M-H-120). From ^{1}H-NMR, ESI-MS spectral data and by comparison to published data compound **4** could be identified as isovitexin. It was also identified by UPLC/MS/MS peak (9). Isovitexin has also been shown to have anti-oxidant, anti-inflammatory, anti-Alzahimar properties and antiviral activity similar to that of its isomer vitexin **[32]**.

Compound 4: (Apigenin-7-O-β-D glucoside)

White powder soluble in methanol compounds 5 (flavone) was identified as flavonoidal glucoside. UV showed bathochromic shift with NaOMe in band I indicate the presence of hydroxyl group at C-4' but no bathochromic shift with NaOAc/H3 BO3 indicated the absence of hydroxyl group at C-3' and occupied hydroxyl group at C-7. The presence of singlet proton at δ 6.92 ppm (H-3) confirmed the flavone nature of compound 5. Signals at δ 5.01, δ 5.13 ppm (H-1") and δ 3.5-4.15, δ 3.223.91 ppm (H-2", 3", 4", 5" and 6") confirmed the presence of β-D glucose at C-7 for compounds 5. 13C-NMR showed anomeric carbons at 99.13and 103.19 (C-1"). The compound 5 confirmed to be apigenin-7-O-β-D glucoside. That is confirmed by UPLC/MS/MS peak (12) **[33] ESI/MS: 431,269,**227

Antimicrobial Activity

Beta vulgaris extract showed concentration dependent activity against *C.albicans* and no activity against Gram-positive or Gram-negative bacteria Table (3) Fig (2). As for the novasomes, formula 1 (BV1) showed activity against all tested

organisms at the highest concentration (100 %) and against *E.coli* at (50 %). Formula 2 (BV2) showed concentration dependent activity against bacteria and no activity against yeast.

Table (3): Antimicrobial activity of *Beta vulgaris* and its nano-formulas

Sample	Concentration (%)	*S.aureus*	*E.coli*	*C.albicans*
BV	100	0	0	3.4±3.2
	50	0	0	3±2.8
	25	0	0	0
	12.5	0	0	0
BV1	100	1.1±2	3.5±2.1	1.5±1.9
	50	0	2	0
	25	0	0	0
	12.5	0	0	0
BV2	100	2.2±1.08	2.3±2	0
	50	1.5±2	2±2.5	0
	25	0	1.8±2.3	0
	12.5	0	0	0
DMSO	100	0	0	0
Antibiotic		4.5±2.5	5±1.79	3±2.4

Antioxidant Activity

Beta vulgaris crude extract showed concentration dependent antioxidant activity against the free radical DPPH Table (4), Figure (3). BV1 was the most potent as antioxidant where it inhibited DPPH by 91±2.02 % at concentration 100% followed by BV and BV2

Table (4): Antioxidant activity of *Beta vulgaris* against DPPH. Data are presented as mean±SE, (n = 3).

Sample	Concentration (%)	% DPPH Inhibition
BV	**100**	85± 2.21
	50	40± 1.52
	25	32± 1.9
	IC_{50}	55.2
BV1	**100**	91±2.02
	50	60±1.50
	25	45±1.36
	IC_{50}	33.4
BV2	**100**	58±1.15
	50	32±1.80
	25	13±1.15
	IC_{50}	82.6

Characterization of the selected hyaluronate coated novasomes:

1- **Extract content % measurements, Particle size, zeta, and PDI**

The amount of extract retained within selected coated novasomes is referred as drug content. The drug content of the selected system was 62.03 % **(table 5).** All the results clearly indicated that the novasomes stabilized hyaluronate and prepared with mixed SAA and FAA at percent of 2:1 of (span 60: Oleic acid) respectively could effectively nanoencapsulate the extract after preparation.

The results of zeta potential, particle size, PDI of the prepared novasomes are presented in **table (5).** Particle size value was in the nanorange (340.2 nm). Value of the zeta potential often indicate how stable the nanoparticles are **[34]** The zeta potential value was negative. This could be due to the anionic nature of sodium hyaluronate. The selected formulation zeta potential values are higher than – 30 suggesting the high stability of the prepared systems. PDI value was below 0.5 indicating narrow size distribution. So, Formulation (F1) showed high EE, high zeta potential and low PDI values, reasonable particle size suggesting it as a successful system to encapsulate the extract.

2-***TEM***

The morphological structure of the selected formulation (F1) was visualized by TEM **(figure 4).** They were spherical in shape and free from aggregations. The nanovesicles showed very homogenous morphology with porous surface including tiny dark stained dotes referring to the extract successful enclosing in the selected nano system cavities plus to its quite uniform particle size distribution. The particle size diameters obtained by TEM imaging were around 91.49 nm. The average hydrodynamic size (**Table 5)** determined by the DLS particle size analyser was larger than the TEM images because of the tendency of the nano coated vesicles to aggregate. TEM images offer lower aggregation of nanoparticles and easier observation of the size and shape of nanoparticles.

Table (5): PS, ZP and PDI of the selected hyaluronate coated novasomes

Formulation	Drug content (%)	PS (nm)±SD	ZP (mV)± SD	PDI
F1 coated	62.03 ±0.12	340.2±1.58	-34.05 ±3.28	0.455

3. FTIR

Figure (5) displays the FTIR spectra of extract and extract-loaded formulation F1. The reported characteristic peaks of extract come in agreement with the present results suggesting the purity of the extract under investigation. The formation of novasomes loaded with extract is confirmed by the FTIR spectra of the formulation F1 comparing the spectra of the crude pure extract with the spectra of F1. FTIR spectra showed changes in peaks intensity and position of extract after inclusion in F1. The successful development of these nano-systems and the beneficial substance encapsulated within them may be demonstrated by the decrease in the band's intensity of the prepared nanovesicles and the elimination of other bands.

Disscussion

The current study targeted to investigate the phytochemical composition of aerial parts of *Beta vulgaris* subsp. *Cicla* ethanol extract and its hyaluronate coated novasomes as antimicrobial agent.The previous studies suggested the antimicrobial

properties of flavonoids, phenolic compounds, and flavonoid glycosides, predominantly present in *Beta vulgaris* subsp. *Cicla*, can be attributed to their interaction with bacterial membrane proteins or lipids. This interaction leads to the stabilization and disruption of the bacterial membrane, inhibition of membrane-bound enzymes, modulation of the immune response, and disruption of lipid rafts. Conversely, another recognized mechanism of the antibacterial activity of kaempferol and quercetin involves membrane permeabilization and rupture. This process is facilitated by an increase in bacterial membrane permeability, interference with bacterial energy metabolism, and a reduction in pathogenicity, which ultimately results in cytoplasmic leakage **[35].**

Mohammed et al. **[36]** isolated five flavone C-glycosides from n-butanol extract of *B. vulgaris* subspecies *cicla* L namely; (vitexin) showed a promising antibacterial activity, 2"-Oxylopyranosylvitexin, acacetin 8-C-β-D-glucopyranoside, acacetin 8-C-α-L-rhamnoside , and vecinin-II. also confirmed that the plant could be used as a natural source of antibiotic.

Numerous studies have assessed the extract from *B. vulgaris* subsp. *crassa* was found to have low effectiveness against breast cancer but potential cytotoxicity against cervical and prostate cancer. Furthermore, it has been demonstrated that the plant extracts are highly efficient against infection-causing bacterial and fungal species **[37].**

Agar well diffusion method was used to test the potential of BV extract and its novasomes (BV1 and BV2) as antimicrobial on *S.aureus*, *E. coli* and *C.albicans.* The crude extract showed activity against *C.albicans* and no activity against both tested bacteria. Meanwhile, BV1 exhibited antimicrobial activity against both *S.aureus* and *E. coli* in addition to *C.albicans*. As for BV2, it possessed antibacterial activity against tested bacteria only.

Several studies have been conducted on the antimicrobial activity of *Beta vulgaris*. Saani et al. **[5]** tested the methanolic and ethanolic extract of *Beta vulgaris* against Gram-positive and Gram-negative bacteria where both extracts possessed antibacterial activity against tested bacteria. Beet root peel methanolic extract was found to possess antimicrobial activity against *E. coli, S.aureus, K.pneumonia and S.flexneri* this indicates that it can be employed in functional food **[38].**

DPPH (1,1-diphenyl-2-picrylhydrazl) is a stable nitrogen free radical which changes its colour from purple to yellow upon being neutralized by potent antioxidants **[39].**

Upon testing *Beta vulgaris* crude extract and its novasomes for their antioxidant activity using DPPH assay, they were found to have antioxidant potential that increases with increasing the concentration. BV1 showed the highest activity with IC_{50} 33.4% followed by the crude extract (IC_{50} 55.2%).

Beside exerting antimicrobial activity, Beta vulgaris have shown antioxidant activity according to previous studies**.** John et al. **[38]** compared methanol and acetone extracts of beet root regarding their antioxidant activity. It was found that both exerted activity where methanol extract showed more potency than acetone one**.** Čeryová et al. **[4]**compared total phenolic content (TPC) in different parts of *Beta vulgaris* and correlated it with their antioxidant activity. It was found that TPC of leaves was higher than stems accordingly, the antioxidant activity of leaves was higher than stems**.**

Formula BV1 showed remarkable antioxidant activity than formula BV2 as it contains double the amount of span 60. Span 60 is not an antioxidant itself, but it can enhance the antioxidant properties of other compounds by acting as a surfactant in drug delivery systems to improve their delivery and stability. By forming these delivery systems, Span 60 helps protect and deliver potent antioxidant molecules, such as folic acid, increasing their effectiveness in various applications **[40]**. Also span 60 has no inherent antimicrobial activities but it is reported that upon using it in drug delivery systems it enhancing the antimicrobial activities of the antimicrobial drugs **[41]** This comes in agreement with our results.

Conclusion:

This study introduces a novel and eco-innovative strategy to combat microbial infection by developing hyaluronate coated novasomes with ethanolic extract of *beta vulgaris subsp. Cicla arial parts* . The formulation of choice showed remarkable encapsulation efficiency , reasonable particle size and zeta potential. FTIR spectra showed absence of some peaks in the spectra of the selected formulation meaning successful encapsulation of the extract in the novasomes. The morphology showed complete spherical novasomes with a coating layer of hyaluronate. The findings of the research revealed that ethanol *Beta vulgaris subsp. Cicla* arial parts extract exhibited remarkable antimicrobial) and antioxidant capabilities (extract and selected hyaluronate coated novasomes). The hyaluronate coated novasomes emerges as a promising candidate for future development as a safe, effective, and environment friendly therapeutic intervention for antimicrobial therapy.

This work underscores the therapeutic and industrial potential of nanotechnology-based delivery of botanical actives for sustainable skin health solutions. the crude extract showed antimicrobial activity against *C.albicans* only while novasomes formulations BV1 was active against both bacteria and yeast. As for BV2, it was active against bacteria only So BV1 was selected for characterization.

Materials and Methods

I. Phytochemical investigation

Plant Material

Fresh arial parts of *Beta vulgaris* subsp. *cicla.* were collected from local market, Giza, Egypt in January 2023**.** The identification of the plant material was confirmed by Ms. Therese Labib, Botanical specialist and consultant Orman Botanical Garden, a voucher specimen

(M 275) was deposited at Pharmacognosy Dept., National Research Centre, Cairo, Egypt. The collected arial parts (3 Kg), was air dried, powdered and kept in tightly-closed containers.

1.2. Preparation of the extract

The dried powdered arial parts of *Beta vulgaris* subsp. *cicla* have been extracted with ethanol then extracted with dicloromethane each extract concentrated by using rotary evaporator, dried and kept in well closed containers.

Chemical evaluation of the dichloromethane fraction using GC/MS

At the Department of Medicinal and Aromatic Plants Research, National Research Center and under the subsequent conditions, the dichloromethane fraction was subjected to GC/MS analysis using a gas chromatography-mass spectrometry instrument presented: TG-WAX MS column (30 m x 0.25 mm i. d., 0.25 µm film thickness), coupled with an ISQ Single Quadrupole MS Spectrometer as detector, and a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA). Helium was employed as a carrier gas with a flow rate of (1.0 mL/min) using the following temperature program: 60 °C for 1 minute, followed by a rise of 4 °C/min to 300 °C and a 15-minute rest. Both the injector and the detector had a temperature of 280°C. At 70 eV, MS spectral measurements were collected using (EI). The analytical approach of mass spectra (genuine chemicals, Wiley spectral library collection, and NSIT library) was used to recognize the substances.

LC-ESI-MS/MS

Instrument

The analysis of the sample was performed using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an ExionLC AC system for separation and SCIEX Triple Quad 5500+ MS/MS system equipped with an electrospray ionization (ESI) for detection in chromatography laboratory at Center of Scientific Excellence, National Research Center

Method of LC-ESI-MS/MS in negative ionization modes:

a Ascentis® Express 90 Å C18 Column (2.1×150 mm, 2.7 µm) was used for separation. Two eluents, A: 5 mM ammonium formate pH 8 (-ve) and B: acetonitrile (LC grade), made up the mobile phase. 5% B at 0-1 min, 5-100% B from 1-20 min, 100% B from 20-25 min, 5% at 25.01, and 5% from 25.01-30 min were the planned mobile phase gradients. The injection volume was 5 µl, and the flow rate was 0.3 ml/min. Negative ionization mode was used for MS/MS analysis using a scan (EMS-IDA-EPI) for MS1 between 100 and 1000 Da with the following parameters: 25 psi of curtain gas IonSpray voltage: -4500 (-ve); source temperature: 500°C; ion source gases 1 and 2 were between 50 and 1000 Da for MS2 with a declustering potential of -80; collision energy: -35 (-ve).

Isolation and identification of the major compounds

The dichloromethane fraction was loaded on preparative TLC using benzene - ethyl acetate (8:2 v/v). spraying with 10% H_2SO_4. The colored bands were marked and gathered. Also, the ethanol extract was loaded on preparative TLC using Dichloromethane: methanol (9:1v/v). The plates were examined under the UV light at 254 nm and 365 nm The Rf values of the isolated compounds were determined. The structure elucidation of some the obtained compounds was confirmed by different spectral analyses (H1-NMR, C13-NMR and MS)

Preparation of sodium hyaluronate coated novasomes:

Oleic acid, span 60 (Sorbitan monostearate) and cholesterol were obtained from sigma Aldrich Co. USA, Sodium hyaluronate (molecular weight 90–100 kDa) was obtained from Shanghai Yuanye Bio-Technology Co., China. Tego care 450 (polyglyceryl-3 methyl glucose distearate) was donated from Goldschmidt (Now Evonik, Essen, Germany). Dichloromethane (HPLC grade) was supplied by Fisher Scientific, UK.

With a few adjustments, the ethanol injection procedure was used to prepare extract-coated novasomes. To manufacture 0.1% (w/w) extract-loaded novasomes, the extract was dissolved in dichrolomenthane and injected into a water phase that contained 10 milliliters of distilled water. Cholesterol (100 mg) and various ratios of surfactants (SAA) (Span 60 and tegocare 450) to oleic acid (FAA) were also added (2:1:1, 1:1:1; respectively). 0.5% sodium hyaluronate **[42]** was magnetically stirred until fully dissolved. Following that, the produced novasomes were magnetically agitated at 1000 rpm

while the sodium hyalorunate solution was added. To reduce variance, the containers were maintained consistent. For stability, the finished product was agitated for a further half hour.

I. Biological investigation methods:

Antimicrobial activity:

Antimicrobial activity of *Beta vulgaris* extract (BV) and its novasomes formulations (BV1, BV2) were tested using agar well diffusion method against, *Staphylococcus aureus* ATCC 6538 as an example of Gram-positive bacteria, *Escherichia coli* ATCC 25922 as an example of Gram-negative bacteria and *Candida albicans* ATCC 10231 as yeast **[43,44,45].** Tested microorganisms were obtained from American Type Culture Collection (Manassas, VA, USA) and maintained on Luria-Bertani agar at 4°C. An inoculum of each organism was made and adjusted to 1.5×10^8 CFU/mL (0.5 McFarland standard) from which (100 µL) were spread on petri dishes containing (20 mL) nutrient agar in case of bacteria or potato dextrose agar in case of yeast **[46]** In each well (0.9 cm), (100 µL) of each sample concentration (100, 50, 25, 12.5 % in DMSO) were placed and incubated for 24 hrs at 37ºC. DMSO (100 µL) was used as negative control while chloramphenicol and nystatin as positive control for bacteria and yeast successively. At the end of incubation period, antimicrobial activity was determined by measuring zone of growth inhibition around the wells in cm **[44]**.

Antioxidant activity

DPPH radical scavenging ability, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was employed to test the antioxidant potential of different concentrations (100, 50 and 25 %) of crude extract (BV), novasome 1 (BV1) and 2(BV2) in DMSO according to **[47]** with some modifications. From each concentration, (100µL) was added to (900µL) of (0.1 mM) DPPH solution in methanol, mixed well and left in dark for 30 min at temperature 37ºC. Percent of decolorization of DPPH was determined spectrophotometrically by measuring the reaction mixture absorbance at 517 nm and applying the following equation:

% Scavenging activity = (DPPH absorbance-Reaction mixture absorbance/DPPH absorbance) x 100

All the tests were performed in triplicate. Activity against DPPH free radical is represented as % inhibition ± SD. One formulation was selected based on the antimicrobial and antioxidant activities for further evaluations.

II. Characterization of the selected formulation:

Extract Content (%):

One gram, of the extract vesicles was taken in order to determine the encapsulation efficiency (EE)%. Dimethyl sulphoxide (DMSO) was used to dissolve the vesicles finely, and they were then agitated for 24 hours using a magnetic stirrer **[48]** samples were withdrawn, filtered, diluted suitably and measured spectrophotometrically **[49]** for extract content at a wavelength of 745 nm. In summary, 1 milliliter was sampled, followed by the addition of 5 milliliters of diluted Folin Catechaeau Reagent (folin/water 1:9 v/v) and (4 mL) of 7.5% sodium carbonate (w/v). After a 24-hour dark incubation phase, the mixture was mixed with a vortex mixing and examined **[50]**.The EE% was defined as the ratio of the quantity of extract entrapped in these generated vesicular systems to the initial extract added content of the formulation. All experiments were performed in triplicates. Encapsulation efficiency (EE) % was calculated using the equation **[51]**:

$$\text{EE\%} = \frac{\text{quantity of extract entrapped}}{\text{Total quantity of extract added}} \text{X100}$$

Particle size, PDI and zeta potential measurements*:* Particle size, PDI, and zeta potential (ZP) were evaluated using photon correlation spectroscopy using the Malvern Zetasizer Nano ZS. The dispersion was doubly diluted with room temperature distilled water and then briefly sonicated in a bath before measurement. Before the sample was put in a quartz cuvette for room-temperature analysis, one milliliter of each vesicular system was appropriately diluted with up to ten milliliters of double-distilled water **[52,53]**.

Transmission electron microscope*:* The morphological characteristics of the selected vesicular system were assessed in order to quantify structural aspects, such as size and shape homogeneity. The selected formulation was diluted with 1:10 bi-distilled water just prior to the investigation. Each sample was placed on a carbon-coated grid with a mesh size of 300 and left to air dry at room temperature. Before the film had completely cured, 1% phosphotungestic acid severely stained it. A TEM (JEOL, JEM-1230, Tokyo, Japan) operating at room temperature and 80 KV of acceleration was used to evaluate the film before micrographs were taken at the proper magnifications.

FTIR*:* An FT-IR spectrophotometer (JASCO 6100, Tokyo, Japan) was used to perform spectral analyses of the extract and the selected lyophilized system in order to identify any potential interactions between the formulation components. Potassium bromide (KBr) and solid samples (the extract and the freeze-dried sample) were combined separately **[40]** and compressed for two minutes at 200 kg/cm^2 pressure to create compact discs. All samples were scanned between 4000 and 400 cm-1 Hz against a blank KBr pellet background **[53]**.

Author Contribution Statement

Doaa A. H. Deabes and Eman A. W. El-abd designed the plant experiment, conducted the assessments, analyzed the data, and wrote the draft of this section. Alaa M. Saleh designed the biological experiment, conducted the assessments, analyzed the data, and wrote the draft of this section. Eman S. Shalaby preparation of novasomes and measurement of the its required parameters and wrote the draft of this section. All authors revised the manuscript and approved the final form for submission.

Acknowledgments
The authors wish to express their gratitude to the facilities provided by the National Research Centre (NRC), Cairo, Egypt.

Funding
No funding is available for this research work.

Data Availability
All data generated or analyzed during this study are included in this article and its supplementary information file.

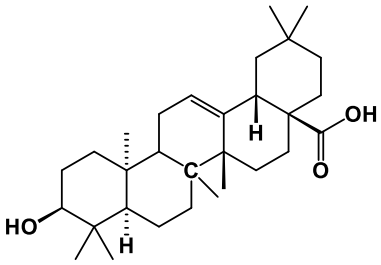
References

1. Parcheta, M., Świsłocka, R., Orzechowska, S., Akimowicz, M., Choińska, R. and Lewandowski, W., 2021. Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials*, *14*(8), p.1984.
2. El-Wahab, A., El-Emary, F.A.A. and Ahmed, M.M., 2022. Comparative botanical studies of some cultivated groups of Beta vulgaris L.(Chenopodiaceae). *Archives of Agriculture Sciences Journal*, *5*(2), pp.96-110.
3. Hashem, A.N., Soliman, M.S., Hamed, M.A., Swilam, N.F., Lindequist, U. and Nawwar, M.A., 2016. Beta vulgaris subspecies cicla var. flavescens (Swiss chard): flavonoids, hepatoprotective and hypolipidemic activities. *Die Pharmazie-An international journal of pharmaceutical sciences*, *71*(4), pp.227-232.
4. Čeryová, N., Lidiková, J., Grygorieva, O., Brindza, J., Demianová, A., Jurčaga, L. and Harangozo, Ľ., 2025. Nutritional Composition, Polyphenol Content, and Antioxidant Activity of Swiss Chard (Beta vulgaris L. subsp. cicla). *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *9*(1).
5. Saani, M. and LAwRENCE, R.E.E.N.A., 2020. Beta vulgaris root extracts: as free radical scavengers and antibacterial agent. *Oriental Journal of Chemistry*, *36*(4).
6. Trifunovic, S., Topalovic, A., Knezevic, M. and Vajs, V., 2015. Free radicals and antioxidants: antioxidative and other properties of Swiss chard (Beta vulgaris L. subsp. Cicla). *Poljoprivreda i Sumarstvo*, *61*(2), p.73.
7. Gamba, M., Raguindin, P.F., Asllanaj, E., Merlo, F., Glisic, M., Minder, B., Bussler, W., Metzger, B., Kern, H. and Muka, T., 2021. Bioactive compounds and nutritional composition of Swiss chard (Beta vulgaris L. var. cicla and flavescens): a systematic review. *Critical reviews in food science and nutrition*, *61*(20), pp.3465-3480.
8. Liu, R., Choi, H.S., Zhen, X., Kim, S.L., Kim, J.H., Ko, Y.C., Yun, B.S. and Lee, D.S., 2020. Betavulgarin isolated from sugar beet (Beta vulgaris) suppresses breast cancer stem cells through Stat3 signaling. *Molecules*, *25*(13), p.2999.
9. Sacan, O. and Yanardag, R., 2010. Antioxidant and antiacetylcholinesterase activities of chard (Beta vulgaris L. var. cicla). *Food and chemical toxicology*, *48*(5), pp.1275-1280.
10. Abd El-Ghffar, E.A., Hegazi, N.M., Saad, H.H., Soliman, M.M., El-Raey, M.A., Shehata, S.M., Barakat, A., Yasri, A. and Sobeh, M., 2019. HPLC-ESI-MS/MS analysis of beet (Beta vulgaris) leaves and its beneficial properties in type 1 diabetic rats. *Biomedicine & Pharmacotherapy*, *120*, p.109541.
11. Mosallam, S., Ragaie, M.H., Moftah, N.H., Elshafeey, A.H. and Abdelbary, A.A., 2021. Use of novasomes as a vesicular carrier for improving the topical delivery of terconazole: in vitro characterization, in vivo assessment and exploratory clinical experimentation. *International journal of nanomedicine*, pp.119-132.
12. Zamboni, F., Okoroafor, C., Ryan, M.P., Pembroke, J.T., Strozyk, M., Culebras, M. and Collins, M.N., 2021. On the bacteriostatic activity of hyaluronic acid composite films. *Carbohydrate polymers*, *260*, p.117803.
13. Bonechi, C., Tamasi, G., Donati, A., Leone, G., Consumi, M., Cangeloni, L., Volpi, V., Magnani, A., Cappelli, A. and Rossi, C., 2021. Physicochemical characterization of hyaluronic acid and chitosan liposome coatings. *Applied Sciences*, *11*(24), p.12071.
14. Kaul, A., Singampalli, K.L., Parikh, U.M., Yu, L., Keswani, S.G. and Wang, X., 2022. Hyaluronan, a double-edged sword in kidney diseases. *Pediatric Nephrology*, *37*(4), pp.735-744.
15. Elmotasem, H., Salama, A.A. and Shalaby, E.S., 2024. Hyaluronate functionalized Span-Labrasol nanovesicular transdermal therapeutic system of ferulic acid targeting diabetic nephropathy. *International Journal of Biological Macromolecules*, *279*, p.135292.

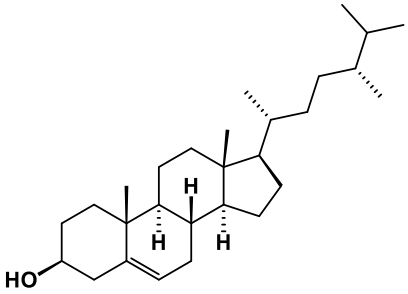
16. Almeida, D., Petropoulos, S.A., da Silveira, T.F., Pires, T.C., Ferreira, I.C., Fernandes, Â. and Barros, L., 2025. Exploring the Biochemical Profile of Beta vulgaris L.: A Comparative Study of Beetroots and Swiss Chard. *Plants*, *14*(4), p.591.
17. El Sayed, A.M., El-Abd, E.A., Afifi, A.H., Hashim, F.A., Kutkat, O., Ali, M.A., El Raey, M.A. and El Hawary, S.S., 2024. Comparative metabolomics analysis of Citrus medica var. sarcodactylis Swingle and Limonia acidissima Linn. Fruits and leaves cultivated in Egypt in context to their antiviral effects. *Heliyon*, *10*(11).
18. Nossier, F.H., Abdelmonem, A.R., Ashour, R., Abdel-Aziz, M., Al_karmalawy, A. and Elsayed, A., 2025. UPLC-QTOF-MS/MS Based Identification of Bioactive Compounds from Garcinia livingstonei Leaves and Evaluation of their Antioxidant and Antiarthritic Activities. *Egyptian Journal of Chemistry*, *68*(2), pp.243-261.
19. Elgendi, S., Ezzat, M. and Elsayed, A., 2023. HPLC-PDA-ESI-MS-MS analysis of acids content of Lantana camara L. flower extract and its anticoagulant activity. *Egyptian Journal of Chemistry*, *66*(1), pp.249-256.
20. Bystrom, L.M., Lewis, B.A., Brown, D.L., Rodriguez, E. and Obendorf, R.L., 2008. Characterisation of phenolics by LC–UV/Vis, LC–MS/MS and sugars by GC in Melicoccus bijugatus Jacq.'Montgomery'fruits. *Food chemistry*, *111*(4), pp.1017-1024.
21. Abd-El-Aziz, N.M., Hifnawy, M.S., Lotfy, R.A. and Younis, I.Y., 2024. LC/MS/MS and GC/MS/MS metabolic profiling of Leontodon hispidulus, in vitro and in silico anticancer activity evaluation targeting hexokinase 2 enzyme. *Scientific Reports*, *14*(1), p.6872.
22. Boso Alonso, S., Gago Montaña, P., Santiago Blanco, J.L., Martínez-Bartolomé, M.A. and Martínez Rodríguez, M.D.C., 2022. Polyphenols in the Waste Water Produced during the Hydrodistillation of 'Narcea Roses' Cultivated in the Cibea River Valley (Northern Spain).
23. Hassan, W.H., Abdelaziz, S. and Al Yousef, H.M., 2019. Chemical composition and biological activities of the aqueous fraction of Parkinsonea aculeata L. growing in Saudi Arabia. *Arabian journal of chemistry*, *12*(3), pp.377-387.
24. Mustafa, A.M., Abouelenein, D., Angeloni, S., Maggi, F., Navarini, L., Sagratini, G., Santanatoglia, A., Torregiani, E., Vittori, S. and Caprioli, G., 2022. A new HPLC-MS/MS method for the simultaneous determination of quercetin and its derivatives in green coffee beans. *Foods*, *11*(19), p.3033.
25. Deabes, D.A., El-Abd, E.A., Baraka, S.M., El-Gendy, Z.A., Korany, R.M. and Elbatanony, M.M., 2025. Metabolomics analyses and comparative insight to neuroprotective potential of unripe fruits and leaves of Citrus aurantium ethanolic extracts against cadmium-induced rat brain dysfunction: involvement of oxidative stress and akt-mediated CREB/BDNF and GSK3β/NF-κB signaling pathways. *Metabolic Brain Disease*, *40*(1), p.89.
26. El-Feky, A.M., El-Abd, E.A., Deabes, D.A. and El-Rashedy, A.A., 2025. Phytochemical investigation and in vitro biological evaluation of Beta vulgaris leaves as anti-diabetic, anti-Alzheimer's and anti-inflammatory agents with computer-guided docking study. *Discover Applied Sciences*, *7*(9), p.1040.
27. Shaker, K.H., Zohair, M.M., Hassan, A.Z., Sweelam, H.T.M. and Ashour, W.E., 2022. LC–MS/MS and GC–MS based phytochemical perspectives and antimicrobial effects of endophytic fungus Chaetomium ovatoascomatis isolated from Euphorbia milii. *Archives of microbiology*, *204*(11), p.661.
28. Wang, J., Jayaprakasha, G.K. and Patil, B.S., 2020. UPLC-QTOF-MS fingerprinting combined with chemometrics to assess the solvent extraction efficiency, phytochemical variation, and antioxidant activities of Beta vulgaris L. *Journal of Food and Drug Analysis*, *28*(2), p.217.
29. Castellano, J.M., Ramos-Romero, S. and Perona, J.S., 2022. Oleanolic acid: extraction, characterization and biological activity. *Nutrients*, *14*(3), p.623.
30. Verma, S.C., Jain, C.L., Nigam, S. and Padhi, M.M., 2013. Rapid extraction, isolation, and quantification of oleanolic acid from Lantana camara L. roots using microwave and HPLC-PDA techniques. *Acta Chromatographica*, *25*(1), pp.181-199.
31. Uttu, A.J., Sallau, M.S., Ibrahim, H. and Iyun, O.R.A., 2023. Isolation, characterization, and docking studies of campesterol and β-sitosterol from Strychnos innocua (Delile) root bark. *Journal of Taibah University Medical Sciences*, *18*(3), pp.566-578.
32. He, M., Min, J.W., Kong, W.L., He, X.H., Li, J.X. and Peng, B.W., 2016. A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia*, *115*, pp.74-85.
33. Abou Zeid, E.M., Ghani, A.E.A., Mahmoud, M.Y. and Abdallah, R.H., 2022. Phytochemical investigation and biological screening of ethyl acetate fraction of Salvia hispanica L. Aerial parts. *Pharmacognosy Journal*, *14*(1).
34. Sankhla, A., Sharma, R., Yadav, R.S., Kashyap, D., Kothari, S.L. and Kachhwaha, S., 2016. Biosynthesis and characterization of cadmium sulfide nanoparticles–an emphasis of zeta potential behavior due to capping. *Materials Chemistry and Physics*, *170*, pp.44-51.

35. Gumisiriza, H., Olet, E.A., Mwikali, L., Akatuhebwa, R., Omara, T., Lejju, J.B. and Sesaazi, D.C., 2024. Antibacterial and antioxidant activities of flavonoids, phenolic and flavonoid glycosides from Gouania longispicata leaves. *Microbiology Research*, *15*(4), pp.2085-2101.
36. Mohammed, H.S., Abdel-Aziz, M.M., Abu-Baker, M.S., Saad, A.M., Mohamed, M.A. and Ghareeb, M.A., 2019. Antibacterial and potential antidiabetic activities of flavone C-glycosides isolated from Beta vulgaris subspecies cicla L. var. flavescens (Amaranthaceae) cultivated in Egypt. *Current Pharmaceutical Biotechnology*, *20*(7), pp.595-604.
37. Zöngür, A., 2024. Antimicrobial, Antioxidant and Cytotoxic Effects of Essential Oil, Fatty Acids and Bioactive Compounds of Beta vulgaris var. crassa (Fodder Beet). *Indian Journal of Microbiology*, *64*(2), pp.719-731.
38. John, S., Monica, J., Priyadarshini, S., Sivaraj, C. and Arumugam, P., 2017. Antioxidant and antibacterial activities of beta vulgaris l. peel extracts. *Int. J. Pharma Res. Health Sci*, *5*(6), pp.1974-79.
39. Gulcin, İ. and Alwasel, S.H., 2023. DPPH radical scavenging assay. *Processes*, *11*(8), p.2248.
40. Shalaby, E.S. and Shalaby, S.I., 2022. Optimization of folic acid Span 60-organogel to enhance its in vitro and in vivo photoprotection: a comparative study. *Therapeutic delivery*, *13*(11), pp.517-530.
41. Najafian, S., Marsusi, F. and Mirabbaszadeh, K., 2024. Martini 3 coarse-grained models of the niosomes based on Span60 and Tween60. *Journal of Molecular Liquids*, *410*, p.125624.
42. Elmotasem, H., Salama, A.A. and Shalaby, E.S., 2024. Hyaluronate functionalized Span-Labrasol nanovesicular transdermal therapeutic system of ferulic acid targeting diabetic nephropathy. *International Journal of Biological Macromolecules*, *279*, p.135292.
43. Barry, A.L., 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations. *Antibiotics in laboratory medicine*, pp.1-26.
44. Mostafa, F.A., Abd El Aty, A.A., Hamed, E.R., Eid, B.M. and Ibrahim, N.A., 2016. Enzymatic, kinetic and anti-microbial studies on Aspergillus terreus culture filtrate and Allium cepa seeds extract and their potent applications. *Biocatalysis and Agricultural Biotechnology*, *5*, pp.116-122.
45. Dharmaraj, D., Krishnamoorthy, M., Rajendran, K., Karuppiah, K., Annamalai, J., Durairaj, K.R., Santhiyagu, P. and Ethiraj, K., 2021. Antibacterial and cytotoxicity activities of biosynthesized silver oxide (Ag2O) nanoparticles using Bacillus paramycoides. *Journal of Drug Delivery Science and Technology*, *61*, p.102111.
46. McFarland, J., 1907. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association*, *49*(14), pp.1176-1178.
47. Zhao, G.R., Xiang, Z.J., Ye, T.X., Yuan, Y.J. and Guo, Z.X., 2006. Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. *Food chemistry*, *99*(4), pp.767-774.
48. Zhang, X.Z., Tian, F.J., Hou, Y.M. and Ou, Z.H., 2015. Preparation and in vitro in vivo characterization of polyelectrolyte alginate–chitosan complex based microspheres loaded with verapamil hydrochloride for improved oral drug delivery. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *81*(3), pp.429-440.
49. Kamel, R., Elmotasem, H., Abdelsalam, E. and Salama, A., 2021. Lepidium sativum seed oil 3D nano-oleogel for the management of diabetic wounds: GC/MS analysis, in-vitro and in-vivo studies. *Journal of drug delivery science and technology*, *63*, p.102504.
50. Mostafa, D.M., Kassem, A.A., Asfour, M.H., Al Okbi, S.Y., Mohamed, D.A. and Hamed, T.E.S., 2015. Transdermal cumin essential oil nanoemulsions with potent antioxidant and hepatoprotective activities: in-vitro and in-vivo evaluation. *Journal of Molecular liquids*, *212*, pp.6-15.
51. Briones, A.V. and Sato, T., 2010. Encapsulation of glucose oxidase (GOD) in polyelectrolyte complexes of chitosan–carrageenan. *Reactive and Functional Polymers*, *70*(1), pp.19-27.
52. Mende, M., Schwarz, S., Petzold, G. and Jaeger, W., 2007. Destabilization of model silica dispersions by polyelectrolyte complex particles with different charge excess, hydrophobicity, and particle size. *Journal of applied polymer science*, *103*(6), pp.3776-3784.
53. Khalid, A., Ahmed, N., Qindeel, M., Asad, M.I., Khan, G.M. and ur. Rehman, A., 2021. Development of novel biopolymer-based nanoparticles loaded cream for potential treatment of topical fungal infections. *Drug Devel*

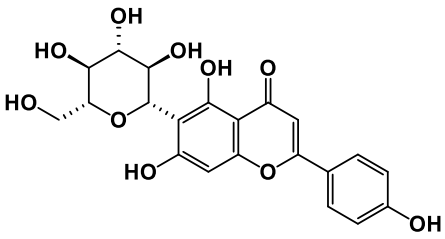
Figures



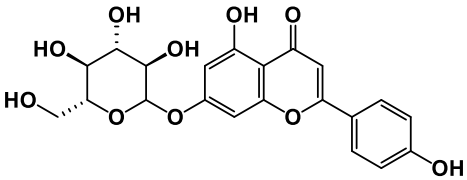
Compound 1: Oleanolic acid



Compound 2: Campesterol



Compound 3: Isovitexin



Compound 4: Apigenin-7-O-D glucoside

Figure (1): Structures of isolated compounds from dichloromethane fraction and total ethanol extract from BV arial parts

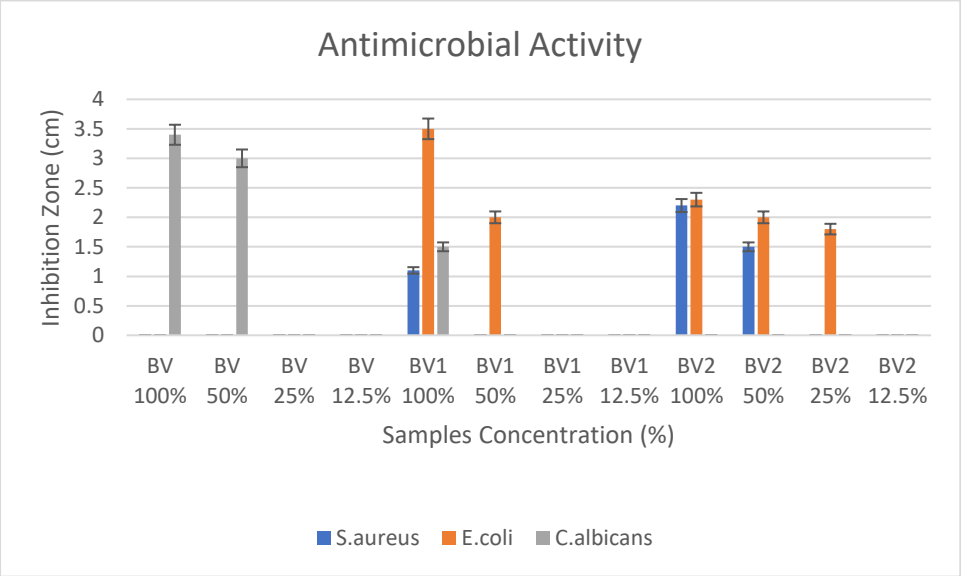


Figure (2). Antimicrobial activity of different concntrations of BV and its nano-formulations. Data are presented as mean±SE, (n=3)

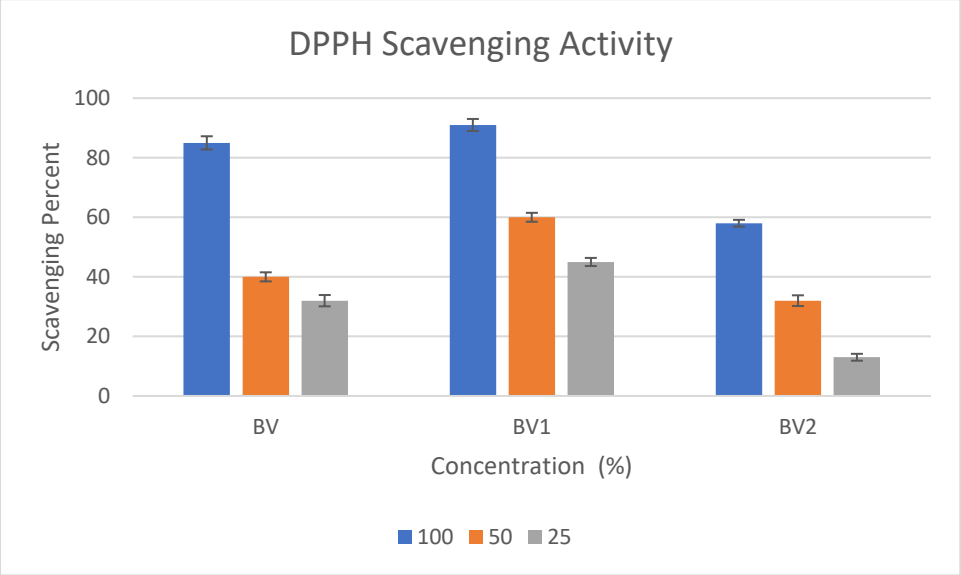


Figure (3): Graph representing the % inhibition of DPPH versus samples. Data are presented as mean±SE, (n = 3)

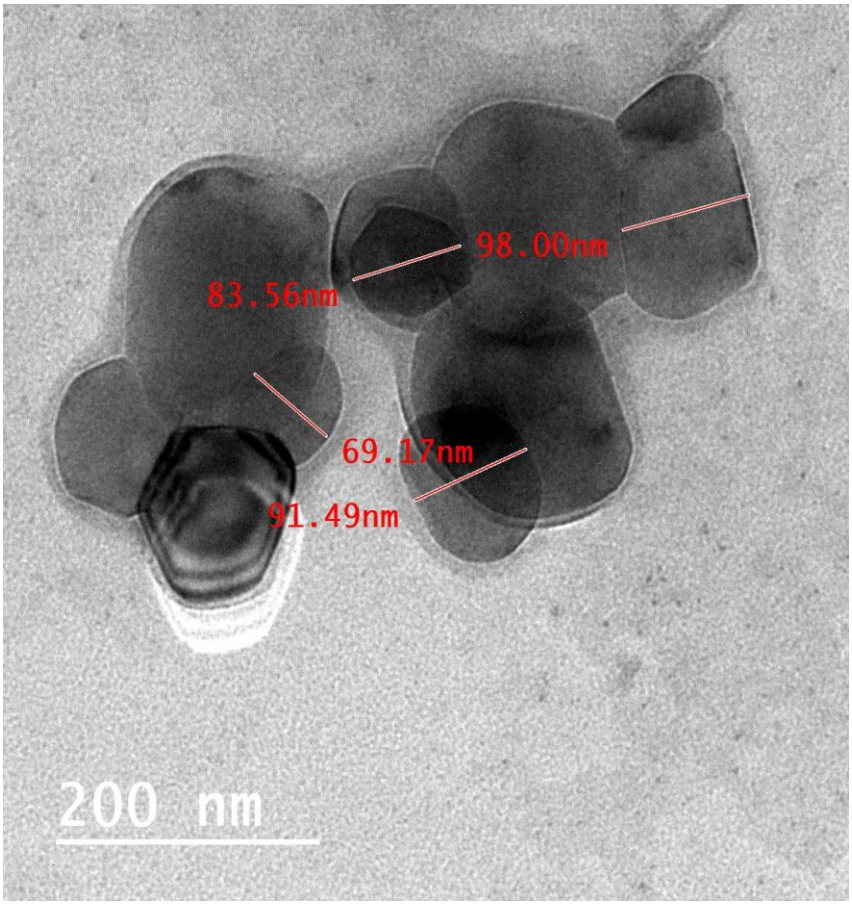
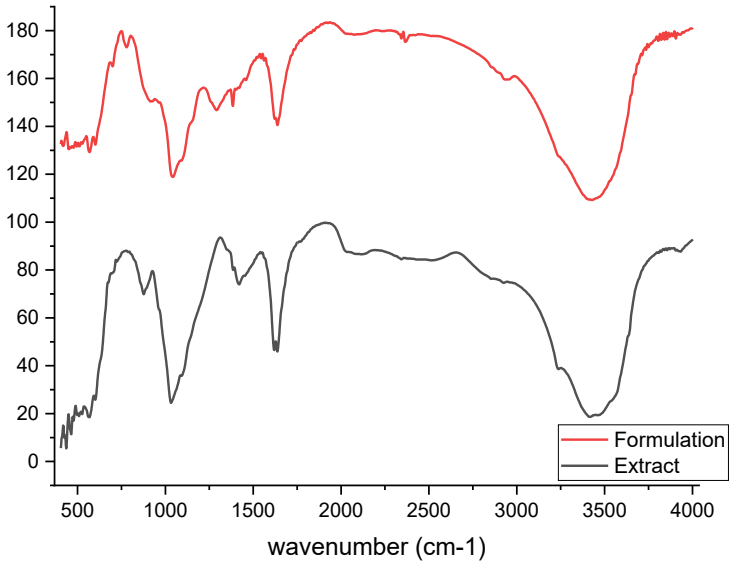


Figure (4): TEM Image of the prepated hyaluronate coated novasomes



561

562 **Figure (5): FTIR spectra of the crude extract and the selected prepared hyaluronate coated novasomes**

563 54. *opment and Industrial Pharmacy*, *47*(7), pp.1090-1099.