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Research Article

Preparation and Evaluation of Phytophospholipid Complex of Phenolic Fractions of *G. glabra* for Antioxidant and Antimicrobial Activity

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Abstract

Objectives: The objective of this study was to evaluate an antioxidant and antimicrobial effect of microencapsulated phyto-phospholipid complex of polyphenolic fractions of *Glycerrhiza glabra*.

Methods: Phenolic fractions of *Glycerrhiza glabra* were incorporated in microencapsulated Phytophospholipid complex. Phytophospholipid complex of phenolic fractions were prepared by rotary evaporation method. Total Phenolic content (TPC) was determined by folin cio-caltechu method. *In-vitro* antioxidant activity of phenolic fractions and phytophospholipid complex of phenolic fractions were determined by DPPH method. Antimicrobial potential of fractions as well as phytophospholipid complex of phenolic fractions were evaluated by agar well method on different seven species of bacteria and fungi.

Results: Total five formulation P1, P2, P3, P4 and P5 of phytophaspholipid complex loaded with phenolic fraction were prepared by rotary evaporation method Amongst five formulation, P2 formulation showed maximum Entrapment efficiency (EE), particle size, zeta potential, drug content and drug release. Antioxidant study revealed that prepared phytophaspholipid complex showed optimum free radical scavenging effect. Antimicrobial study showed that ethyl acetate fraction of *G. glabra* showed zone of inhibition against all the selected microorganism except *P. vulgaris*, Optimized microencapsulated complex P2 formulation showed zone of inhibition against all the species except *P. vulgaris*.

Conclusion: Study revealed that amongst five formulation P2 phytophospholipid complex showed better skin permeation, drug content, sustain release effect, with optimum free radical scavenging effect, and excellent antimicrobial effect than the phenolic fraction.

Keywords: Polyphenols; Free Radical Scavenging Effect; Phytophaspholipid Complex; Antimicrobial Effect

Introduction

Since long time the therapeutic potential of herbal drug proved very popular for the treatment of various diseases. Polyphenolic plant constituents have been proved for their antioxidant activity due to their free radical scavenging potential [1]. They are use in treatment of various diseases in traditional system of medicines as well as in cosmetics. Most of the plant phenolic biomolecules like flavonoid, polyphenols, glycosides are water soluble and large size molecules which limits their therapeutic effectiveness due to poor bioavaibility or absorbance when taken orally or applied topically [2]. Microencapsulated Novel drug delivery system (MNDDS) enhances the drug solubility, absorbance and bioavaibility of biomolecules [3]. Microencapsulated phyto-phospholipid complex (MPPC) can cross lipid rich biomembrane. MPPC produces sustain and control

release-targeted delivery of biomolecules to the site of action. Phytosomes have superior pharmacokinetic and pharmacological parameter. This novel drug delivery system increases the therapeutic potential of biomolecules. *Glycerrihza glabra* is one of the oldest medicinal plant used in treatment of various diseases [4]. It is small perennial herb found in Eurasia, northern Africa, and western Asia commonly known as Licorice, Mulaithi, and sweet wood [5]. Mulethi is well known for its wide therapeutic potential, used in treatment of digestive disorder, respiratory disorders, hyperdipsia, inflammatory disease, epilepsy, fever, sexual debility, paralysis, stomach ulcers, rheumatism, hemorrhagic diseases, jaundice, and skin diseases [6]. Phytoconstituents present in roots and rhizomes are saponins, polysaccharides, glycosides, pectins, simple sugars, asparagines, amino acids, mineral salts, tannins, bitters, essential oil, fat, female hormone estrogen, gums, protein, mucilage, resins, starches, sterols, volatile oils, glycosides, ands, flavonoids, such as glycerhizin, liquirtin, rhamnoliquirilin, liquiritigenin, prenyllicoflavone A, glucoliquiritin apioside, 1-metho-xyphaseolin,shinpterocarpin, shinflavanone, licopyranocoumarin, glisoflavone, licoarylcoumarin, isoangustone A, semilicoisoflavone B, licoriphenone [7,8]. From the research evidence it is found that extract or phenolic fraction of *g. glabra* showed poor absorption because of its poly phenol strucutre (OH) making too polar which cannot cross lipid membrane. limiting their ability to cross lipid bio-membrane results in poor bioavalability [9]. Bioavailability and absorption hurdle can be overcome by formulation it with microencapsulated phyto-phospholipid complex. It is nanovesicular complex of phospholipid and herbal extract to produce fat soluble complex [10]. Phytosomes increase the absorption, bioavaibility and stability of phenolic fractions which increase its therapeutic potency many fold. The aim of present study is to prepare microsphere of phospholipid loaded of phenolic fraction and evaluate for physicochemical properties and antioxidant and antimicrobial effect [11].

Materials and Methods

Roots of *Glycerrhiza glabra* were purchase from the local market. The taxonomic identification was confirmed by the department of pharmacognosy, PWCOP Yavatmal. Roots are dried, chopped and fine powder was prepared.

Chemicals- Soya lecithin, tetrahydrofuran, ethanol, methanol, ethyl acetate, 1,1-diphenyl-2-picrylhydrazyl potassium ferricyanide, sodium nitrite, folin Ciocalteu reagent, sodium nitroprusside. All chemicals of AR analytical grade were purchased.

Preparation of extract and fraction

200 gm of powder of *G. glabra* were extracted by soxhlet apparatus using ethanol for 10 hrs and filtered. The filtrate was evaporated to dryness under pressure. Dissolve dried extract in ethyl acetate and filter. Process were repeated for three times. The filtrate was evaporated under reduced pressure to get polyphenols/ flavonoid rich fraction. Finally, the percentage yield was calculated [12,13].

Determination of total phenolic content

Total phenolic content (TPC) of extract, phenolic fractions and phytosomes loaded with phenolic fraction were determined by Folin-Cio-calteau method. The Folin-Ciocalteu method is used for determination of total polyphenolic compound, based on an electron transfer assay, the reduction of $MoO4^{+}$ to $MoO3^{+}$ that can be measure at 765 nm as change in color from yellow to blue. The principle of Folin–Ciocalteu method is to measure the amount of the substance required to inhibit the oxidation of the reagent, measures the reducing capacity of sample. 1 mg/ml of samples were prepared, mix 1 ml of sample with 1ml of Folin-Cio-calteau reagent. The mixture was kept for 3 min and then 3 ml of sodium carbonate (7.5%) was added, mixed and kept the mixture for 90 minutes with shaking. Then the absorbance was measured at 760 nm and calibration curve was made by using gallic acid as standard. The experiment was performed in triplicate. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dry wt) [14-16].

$$T = \frac{C \times V}{M}$$

Where, T - Total content of phenolic compounds (mg/g of plant extract),

C- The concentration of gallic acid established from the calibration curve(mg/milliliter),

V- The volume of extract (milliliter) and M is the gram weight of plant extract.

Determination of antioxidant activity by DPPH method

Antioxidant activity of extract, phenolic fraction and phytosoes were determined by DPPH method. Free radical scavanging

activity of different extracts, fractions, and isolated compound was determined by DPPH method. DPPH (1, 1 diphenyl-2-picryl-hydrazyl) is stable free radical which do not become unstable on reaction with antioxidant compound. Antioxidant activity of natural compounds can be evaluated by colored methanolic solution of DPPH. Antioxidant biomolecules transfers electron or hydrogen atom to colored DPPH molecule, neutralizes its free radical character and convert it to 1,1-diphenyl-2-picryl-hydrazine. This causes change in color intensity of DPPH solution due to free radical scavenging activity of the extracts/fractions. Antioxidant activity can be determined by measuring change in the absorbance at 517 nm. DPPH solution (0.3 mM) was prepared in ethanol. The concentration of extract, fraction and phytosomes loaded with phenolic fraction diluted with ethanol were taken as 10 ug/ml, 20 ug/ml, 30 ug/ml and 1ml of DPPH solution was added, incubated in dark for 30 min at 37°C. After 30min the absorbance is measured at 517 nm. An equal amount of ethanol with DPPH solution was used as a blank (control) [17-20]. The experiment was performed in triplicate.

The percentage of the DPPH radical scavenging is calculated by the following equation

$$\% \text{ scavenging activity} = \frac{Ac-As}{Ac} \times 100$$

Where Ac- Absorbance of Control

As - Absorbance of Sample

Preparation of phytosomes

Phytosomes were prepared by rotatory evaporation technique

The specific amount of mixture of both drug extract and soya lecithin were dissolved in tetrahydrofuran in rotatory round bottom flask followed by stirring one hour at a temperature not exceeding 40^0C. Thin film of sample was obtained to which n-hexane was added and continuously stirred until monolayered of phospholipid, then phosphate buffer 6.8 added and precipitate was obtained, collected, placed in amber colour bottle at room temperature [21-24]. Total five different P1, P2, P3, P4, P5 phytophospholipid complex were prepared containing soyalecithin and phenolic fraction in the ratio of 1:1, 1:2, 1:3, 2:1, 3:1.

Evaluation of phytosomes

Particle size and zeta potential

Physical stability of phytosomes is determined by Zeta potential which is the most important parameter. The higher the electrostatic repulsion between the particles the greater is the stability. Zeta potential and particle size measurement of the optimized phytosome were done by using the Malvern software. Zeta potential and particle size of optimized phytosomes were determined by injecting the diluted solution into the zeta potential measurement cell [7,21,23].

Optical microscopy

Optical microscopy study was done, one drop of diluted phytosomesloaded with phenolic fraction was taken on glass slide, then slide was allowed to dry and slide was examined under the optical microscope [6,21].

Entrapment efficiency

Entrapment efficacy of phytosomes were evaluated by centrifugation of phytosomes. Phytosomes preparation were centrifuged at 12000 rpm for 60 minutes. Percentage of free drug as the clear supernatant was determined by measuring the absorbance at 420 nm using UV-visible spectrophotometer [7,21].

The % drug entrapment was calculated by following formula.

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of encapsulated drug} \times 100}{\text{Amount of drug added}}$$

Determination of Percentage of yield

% yield of phytosome complex was calculated by the following formula [8,21].

$$\text{Percentage Yield} = \frac{\text{Practical yield} \times 100}{\text{Therotical Yield}}$$

Determination of drug content

Drug content of optimized phyto-phospholipid complex was determined by dissolving 10 mg of phospholipid complex into 10 ml methanol. Absorbance was determined by UV spectrophotometer at 426 nm and drug content was determined [3].

In vitro drug release study

In vitro drug release study of the optimized phytosomes was carried out using USP type II paddle type dissolution apparatus. 0.1N 900 ml HCl was placed into the dissolution flask. The temperature was maintained at 37 ± 0.5^0c with 50 rpm. 100 mg of phytosomes was placed in each chamber of dissolution apparatus. The apparatus was allowed to run for 10 hours. Sample measuring 5 ml were withdrawn after every 1 hour up to 10 hours using 10ml pipette. The fresh dissolution medium was replaced

at every time with the same quantity of the sample. Dilute 0.5 ml of sample upto 10 ml and measured the absorbance at 420.0 nm using spectroscopy [3].

Antimicrobial study

Antimicrobial activity of selected bioactive rich fractions of Liquorice, and phytosomes was performed by the method given by Suzilla W. Y., et al. 2020. 10 mg of each sample was dissolved in 10 ml of sterile distilled water containing 10% dimethyl sulfoxide (DMSO). This resulted in a stock concentration of 1 mg/ml. From this stock, further dilutions were made to 20, 10, 5, and 2.5 (mg/ml) of the samples. Seven strains of micro-organisms (*S. aureus, S. epidermidis, Proteus vulgaris, Bacillus subtilis, Escherichia coli and Aspergillus niger, Candida albicans*) were used to assess the antimicrobial potential of the fractions and formulated phytosomes and pure berberine. Nutrient agar was poured into Petri plates in a laminar airflow cabinet and allowed to solidify. 5 ml of nutrient broth was transferred into each sterile test tube. A loopful two-week-old bacterial, yeast and fungal isolates were inoculated in each 5 ml nutrient broth tube and incubated for 48–72 h. After incubation, the organisms were inoculated using sterile swab sticks and spread on the entire surface of already prepared Mueller-Hinton agar (38 g/l) on culture plates. Wells of 6 mm in diameter were bored into the agar plates using a sterile cork borer. Each concentration of the already diluted samples was transferred into the wells in the volumes of 20 µL, while Amoxicillin 10 mg/L served as a positive control for antibacterial. The plates were then incubated at 37°C for 16-18 h (bacteria) and 48-72 h (for fungi). The results were read by measuring the inhibition zone diameter (IZD in mm) across the wells [26,27].

Results

Total phenolic content-TPC

Name of Sample	% Phenolic Content
Licorice ethanolic extract	6.02
Licorice ethyl acetate fraction	6.04
Phytosomes loaded with phenolic fraction of liquorice	6.044

Table 1: Total phenolic content of ethanol extract, ethyl acetate fraction of G.glabra and phytosomes.

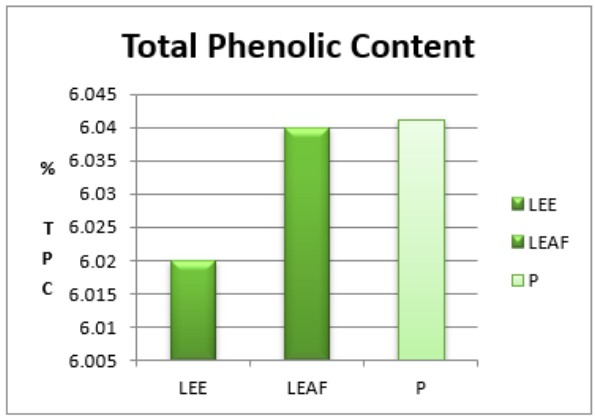


Figure 1: Total Phenolic content Licorice ethanolic extract (LEE), Ethyl acetate fraction of Licorice (LEAF), Phytosomes loaded with phenolic fraction (P).

Antioxidant activity

Name of Sample	Concentration ug/ml	% radica scavenging activity
Liquorice ethanolic extract	10	70.04
	20	72.08
	30	75.55
	40	77.85
	50	80.12
Liquorice ethyl acetate fraction	10	71.02
	20	72.05
	30	75.69
	40	78.86
	50	82.47
Phytosomes loaded with phenolic fraction of *G. glabra*	10	79.06
	20	79.09
	30	80.22
	40	83.21
	50	86.38

Table 2: Antioxidant activity of ethanol extract, ethyl acetate fraction of *G. glabra*, and phytosomes.

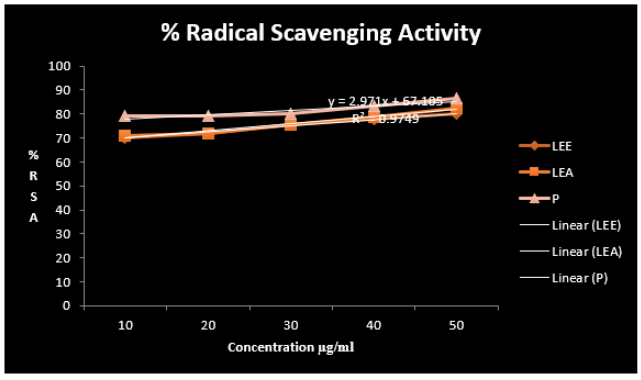
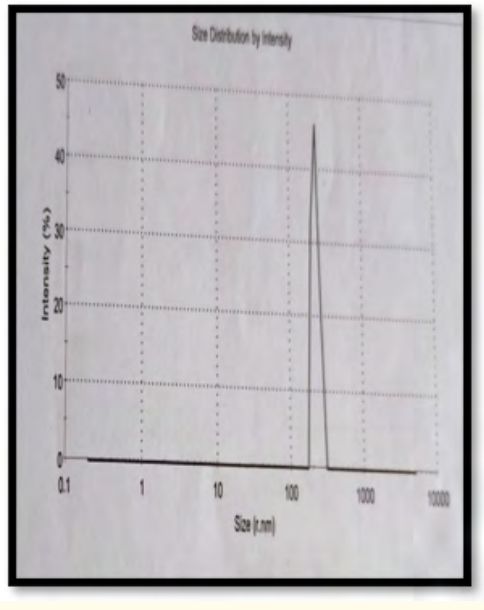


Figure 2: Radical scavenging activity of Licorice ethanolic extract (LEE), Licorice ethyla acetate fraction (LEA), and Phytosomes loaded with phenolic fraction (P).

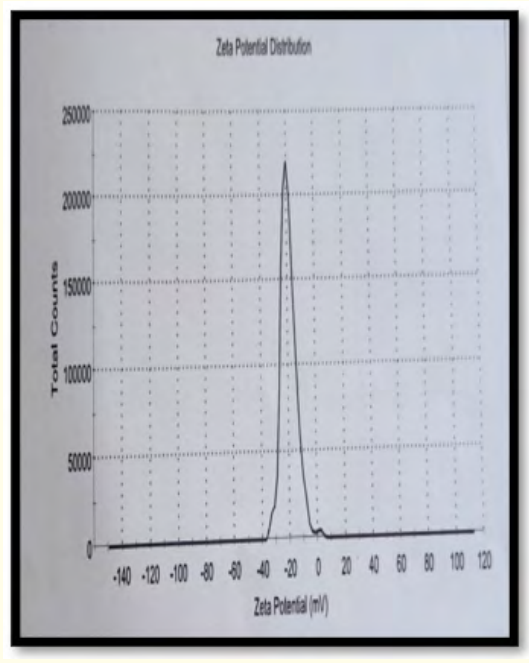
Particle size and zeta potential

Formulation code	Particle size	Zeta Potential
P2	226.7 nm	26.6 Mv

Table 3: Particle size and zeta potential of phytosomes P2 formulation.



Graph 1: Particle size of P2 formulation.



Graph 2: Zeta Potential of P2 formulation.

Entrapment efficiency

Formulation code	Fraction mixture: soya lecithin ration	Entrapment efficiency (%)
P1	1:1	89
P2	1:2	93
P3	1:3	88
P4	2:1	80
P5	3:1	79

Table 4: Entrapment efficiency of Physotomes.

Percentage Yield and Drug content-

% Yield and % Drug content in the optimized phytosomes complex is given in table 5.

Formulation code	% Yield	% Drug content
P1	85	87.86
P2	90.7	89.78
P3	80	86.9
P4	79	85.01
P5	75	80.34

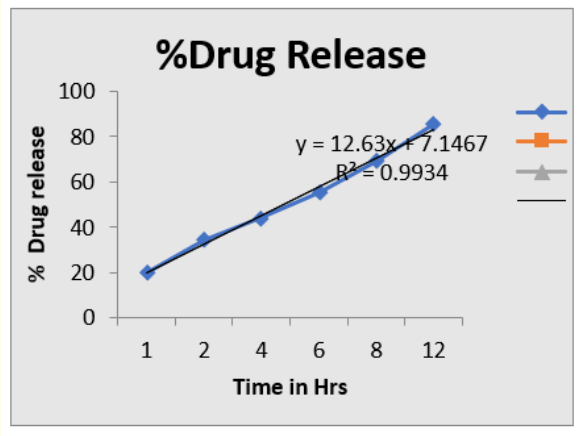
Table 5: Percentage Yield and Percentage Drug content of optimized phytosomes formulation.

Drug release

In-vitro drug release data for P2 formulation shown in table 6.

Time (Hrs)	Log time	Cumulative % of drug release	Log Cumulative % of drug release	Cumulative % of drug remaining	Log Cumulative % of drug remaining
1	0	20.01	1.304	79.99	1.903
2	0.310	34.31	1.526	65.69	1.833
4	0.602	44.24	1.649	55.76	1.644
6	0.778	55.05	1.754	44.95	1.584
8	0.903	69.14	1.912	30.86	1.486
12	1.079	85.36	1.948	14.64	1.124

Table 6: Percentage of drug release of P2 phytosome formulation.



Graph 3: %Drug release of P2 formulation.

In-vitro Antimicrobial activity

Zone of Inhibition in mm at (10 ug/ml, 20 ug/ml).

Discussion

TPC of alcoholic, ethyl acetate fraction of *G. glabra* and optimized phytosomes was found to be 6.02, 6.04, 6.044 respectively. Comparative TPC and antioxidant study revealed that there is strong correlation between PC (Phenolic content) and their free radical scavenging effect. Five different phytophospholipid complex of phenolic fraction mixture and soya lecithin were prepared in a ratio of 1:1, 1:2, 1:3, 2:1, and 3:1. Entrapment efficacy were reported to

Name of sample	*Concentration*	*S. aureus*	*B. subtilis*	*S. epidermidis*	A. Niger	C. albicans	*E. coli*	*P. Vulgaris*
Phenolic fraction of *G. glabra*	10 ug/ml	1 mm	4 mm	1 mm	3 mm	2 mm	1 mm	-
	20 ug/ml	3 mm	5mm	2mm	5mm	4mm	2 mm	-
Phytosomes	10 ug/ml	3 mm	5 mm	2 mm	5 mm	2 mm	2 mm	-
	20 ug/ml	6 mm	6 mm	4 mm	7mm	4 mm	4 mm	
Amoxicillin	10 ug/ml	3 mm	7 mm	5 mm	5 mm	7 mm	7 mm	3 mm

Table 7: Antimicrobial activity of Phenolic fraction of *G. glabra*, and phytosomes.

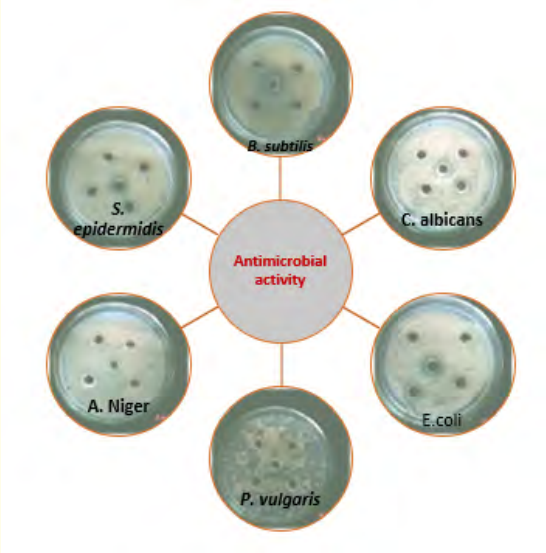


Figure 3: Antimicrobial activity of Phenolic fraction of *G. glabra*, S. cardifolia and *phytosomes*.

be in the range of 79-93%. Prepared phytosomes were compared based on EE, result showed that P2 for mulation phytophospholipid complex with ration 1:2 showed highest entrapement efficiency therefore ratio 1:2 was selected as optimum ratio for evaluation. Antioxidant activity of ethanol extract, ethyl acetate fraction of *G. glabra*, and optimized phytophaspholipid complex was found to be highest at the concentration 50 ug/ml as 80.12%, 82.47%, and 86.38% respectively. Particle size and zeta potential of prepared P2 formulation was found to be 226.7 nm, and 26.6 Mv indicating better stability of phytosomes. *In-vitro* dissolution study indicate that P2 formulation has sustain release dissolution pattern. P2 formulation showed 85.36% of drug release at 12hrs.

Conclusion

Ethyl acetate fraction of *G. glabra* showed optimim phenolic content and antioxidant activity. Microencapsulated phytophaspholipid complex possessed synergistic antioxidant effect. Amongst five formulation, P2 formulation having extract: soya lecithin in the ratio of 1:2 showed optimum EE, drug content and drug release, particle size, zeta potential, antioxidant activity and antimicrobial activity. Study revealed that microencapsulated P2 formulation produced excellent free radical scavainging effect, and antimicrobial effect than the phenolic fraction. This microencapsulated phytophospholipid complex showed better skin permeation, sustain release antioxidant and antimicrobial effect. Thus from the result it is concluded that the phytophospholipid complexes can be a better system for topical delivery of polyphenolic biomolecules in liquorice. Further Invivo antioxidant and antimicrobial study is needed.

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