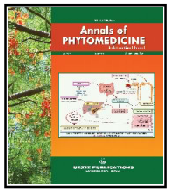
Annals of Phytomedicine 13(1): 667-675, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.68

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

GC-MS analysis and formulation of novel antiulcer phytosomes from methanol extract of *Spondias mangifera* Willd. stem bark

Abdul Baseer Khan, Muhammad Arif◆*, Usama Ahmad, Syed Misbahul Hasan and Mohd. Khushtar
Faculty of Pharmacy, Integral University, Kursi Road, Lucknow-226026, Uttar Pradesh, India

Article Info

Article history
Received 15 December 2023
Revised 25 January 2024
Accepted 26 January 2024
Published Online 30 June 2024

Keywords
Spondias mangifera Willd.
Phytoconstituents
Gas chromatography
Mass spectros copy analysis
Antiulcer activity
Phytosome

Abstract

The bioavailability and therapeutic uses of poor water-soluble natural phytoconstituents are confined. The active compounds of methanolic extract were investigated by the gas chromatography mass spectroscopy (GC-MS) and phytosomes were developed by the thin layer hydration tactic and characterized for their suitable method. The antiulcer activity was assessed employing the indomethacin induced ulcer model in wistar rats. After induction of the ulcer, the test groups *Spondias mangifera* Willd. extract, phytosomes formulation, and positive control ranitidine, received doses (100, 20, and 10 mg/kg) for 7 days. The *S. mangifera*, phytosomes formulation, and ranitidine showed a significant (p<0.05) decrease in ulcer index and thiobarbituric acid reactive substances levels and a remarkable (p<0.05) increase in catalase and superoxide dismutase levels as compared to the negative control indomethacin treated group. The chief phytoconstituents revealed in *S. mangifera* are phenolic compounds like brenzkatechin (0.40%), pyrogallol (88.35%), diethyl phthalate (3.66%), and steroids like gamma sitosterol (0.91). Phytosomes formulation comprises several types of particles, their size increasing with *S. mangifera* concentration, having a zeta potential of -24.24 mV. The present study demonstrated that the phytosome of *S. mangifera* was well prepared and characterized and possessed significant antiulcer activity.

1. Introduction

Ulcers, a common medical problem, are the open sores or breaks in the skin or mucous membranes, affecting millions of people worldwide. The duodenal ulcer may occur on the anterior and posterior walls of the duodenum, characterized by severe pain in the stomach, bloody stool, and a burning sense that makes the patient lethargic (Brooks, 1985). Studies have shown that the Gram-negative bacterium (*Helicobacter pylori*) infection, injury, stress alcoholism, and long-term certain medications like none steroidal anti-inflammatory drugs (NSAIDs) are the real cause of duodenal ulcers. The treatment of ulcers often requires a combination of medications and lifestyle changes, but the search for more effective and targeted treatment options is ongoing (Singh, *et al.,* 2022).

Spndias mangifera Willd., Family Anacardiaceae, is an incredible aromatic tree with curative potential for the treatment of numerous diseases. The plant is normally valued for its turpentinic odor, fruits and flowers which are largely utilized in the preparations of cuisine and the production of pickles (Muhammad *et al*., 2009). The various parts of the plant, *viz.,* young leaves, stem bark, root, fruit, and flowers are used in the folklore medicinal system of different Asian countries and utilized in different ailments conditions such as dysentery, tonsillitis, duodenal ulcer, piles, arthritic pain, helminthiasis, rheumatoid arthritis, cholera, debility, and infections. The bark of this plant is utilized in the treatment of different gastric ailments and is rich in triterpenoids and phenolic compounds (Khan *et al.,* 2022; Arif *et al.,* 2008).

There are a variety of promising therapeutic approaches involving the use of herbal extract formulations, but with the action of digestive secretions at the ulcerated stomach surface, most of the phytoconstituents of traditional herbal formulations are broken down and unable to produce their effects. Many strategies have been recognized to improve the oral bioavailability of herbal phyto-constituents. Phytosomes are lipid based, nano form vesicles of herbal extracts that show better results than traditional herbal extract formulations, which can be employed to improve the antiulcer activity of active phytoconstituents (Usmani *et al.,* 2019; Ajay *et al.,* 2023). The objective of this research was to analyze the phytoconstituents of the stem bark *S. mangifera* by GC-MS, formulate the extract as phytosomes by forming complexes with phospholipid, and compare the antiulcer activity with a simple extract and a standard antiulcer drug.

2. Materials and Methods

2.1 Procurement and certification of plant material

S. mangifera bark was collected in December 2021 from a local area of district Chandouli (India) and validated by the Dept. of Life Science, Dibrugarh University, Assam, to find out the reference number (DLS/MS/2021/14).

2.2 Chemicals and drugs

Thiopental sodium (Sigma-Aldrich), thiobarbituric acid (TBA, Spectrochem), Ranitidine (Jb Chemicals and Pharmaceuticals Ltd.), cholesterol (Geno Pharmaceuticals Pvt., Ltd.) phosphatidylcholine (Mitushi Biopharma), phosphate buffer (prepared as per IP, 2010),

Corresponding author: Dr. Muhammad Arif
Associate Professor, Faculty of Pharmacy, Integral University, Kursi Road, Lucknow-226026, Uttar Pradesh, India
E-mail: arifxyz@iul.ac.in
Tel.: +91-9918464963

and analytical grade chemicals like trichloroacetic acid (TCA), and solvents for extraction were bought from Merck and SD Fine Chemical Limited (Mumbai).

2.3 Instruments

Gas chromatography-mass spectroscopy (Shimadzu QP-2010 Ultra), rotary vacuum evaporator (Buchi Rotavapor R-200), Malvern zeta sizer (Malvern Instrument, Worcestershire, U.K.), and Transmission Electron Microscopy (TEM - Tecnai, G_{20}, Philips Scientific Netherlands).

2.4 Extract preparation of plant materials

The bark (500 g) was screened for extraneous material and dried in the shade for two weeks. The bark powder (200 g) was defatted with Pet. ether (400 ml) and extracted with methanol (400 ml) by the Soxhlet apparatus (Qadir *et al.,* 2018). The methanolic extract of *S. mangifera* (SM) obtained was concentrated and dried to yield 9.35%w/w. The SM (100 mg) was used for GC-MS analysis and 4.0 g extract was used for the preparation of phytosomes nano formulations (SM-PS).

2.5 Gas chromatography-mass spectroscopy

The gas chromatography-mass spectroscopy (GC-MS) technique was used to investigate the phytoconstituents present in SM. The instrument used was Shimadzu QP-2010 Ultra GC-MS with a non-polar column, 60 M TRX 5-MS (Dimension: 30 m, ID: 0.25 mm, film: 0.25 mm) and the reference capillary. The helium gas was used as a vehicle and the mobile phase flow rate was maintained at 1.21 ml per minute. The temperature of ovens was originally raised from 100 to 260°C at a rate of 10°C per min with a fixed injection volume of 2 µl. The electron ionization energy system was 70 eV for the test and the sample was run for 45 min (Kumar *et al.,* 2023).

2.6 Development of *S. mangifera* phytosomes

The SM-loaded phytosomes (SM-PS) were created through a thin layer hydration approach. Ethanol was used to dissolve various amounts of SM, cholesterol, and phosphatidylcholine. The mixture was refluxed and stirred for the specified temperature and time. The final mixture is vacuum evaporated using a vacuum evaporator at 34-45°C until the layer is thin. The films were further hydrated with 90% v/v aqueous ethanol for 6 h and placed in a desiccator overnight. The resulting extract-phospholipid complexes were transferred to vials for further characterization and stored in the refrigerator (Siddiqui *et al.,* 2023).

2.7 Optimization of SM-PS by utilizing Box-Behnken design (BBD)

The SM-PS phytosome formulation was optimized by using the three factors, three-level BBD. As per the design expertise software, 17 run SM-PS phytosome formulations were developed and their corresponding particle size, PDI, and %EE were observed. The independent variables selected were phospholipid (X_1), cholesterol (X_2), and sonication time (X_3) while the dependent variables were vesicle size (Y_1), PDI (Y_2) and percentage entrapment efficiency (Y_3), and constraints for dependent variables are provided in Table 1 (Singh *et al.,* 2005).

Table 1: Experimental runs for SM-loaded phytosomes formulation with independent variables and observed dependent variables

Formulation	Independent variables			Dependent variables		
	X1	X2	X3	Y1	Y2	Y3
1.	40	7.5	180	81.8	0.172	72.5
2.	50	7.5	120	117.8	0.246	84.7
3.	40	7.5	60	91.4	0.185	70.8
4.	50	7.5	120	118.1	0.248	84.2
5.	50	5	60	109.1	0.222	79.1
6.	40	10	120	97.7	0.198	72.5
7.	50	7.5	120	116.9	0.249	84.3
8.	50	10	60	140.6	0.323	84.9
9.	50	7.5	120	117.4	0.241	84.2
10.	60	7.5	180	169.1	0.419	90.2
11.	50	10	180	125.2	0.312	86.2
12.	60	5	120	165.9	0.383	89.9
13.	50	7.5	120	117.3	0.239	84.9
14.	40	5	120	79.5	0.143	62.7
15.	50	5	180	104.7	0.216	77.7
16.	60	10	120	180.6	0.433	93.4
17.	60	7.5	60	175.3	0.43	91.6

X_1-phospholipid (mg), X_2-cholesterol (mg), X_3-sonication time (second), Y_1-vesicle size (nm), Y_2-PdI and Y_3-% EE.

2.8 Characterization of SM-PS

2.8.1 Determination of particle size and polydispersity index (PDI)

The estimation of vesicle size and PDI of the SM-loaded phytosomes was performed using the Malvern zeta sizer (Malvern Instrument, Worcestershire, U.K.) at 25 ± 1°C. The formulation was diluted using pH 7.4 phosphate buffer and determinations were performed in triplicate (Qadir *et al.,* 2020).

2.8.2 Determination of zeta potential

At 25 ± 1°C, the zeta potential of the SM-PS phytosome was measured using a Malvern zeta sizer (Malvern Instrument, Worcestershire, U.K.). The formulations were watered down with Milli-Q water for the analysis was conducted in triplicate (Singh *et al.,* 2011).

2.8.3 Determination of entrapment efficiency (%EE)

The ultracentrifugation approach was employed to determine the %EE of the prepared SM-PS phytosome formulation. For the separation of free SM from the SM-PS phytosome SM-PS phytosome was ultra centrifuged at 15000 rpm for 1 h at 4°C. The different diluted concentrations of the collected SM supernatants were determined using UV spectroscopy at a wavelength of 416 nm. To obtain the percentage of entrapment efficiency, the equation used as:

$$\% \text{ EE} = \frac{\{\text{Iint} - \text{Ssup.}\}}{\text{Iint.}} \times 100$$

where I_{int} is the initial quantity of SM and S_{sup} is the quantity of SM in the supernatant. To summarize, drug quantification was carried out utilizing UV spectroscopy at 416 nm (Sharma *et al.,* 2022).

2.8.4 Transmission electron microscopy (TEM)

A TEM was used to examine the morphology of phytosomes that had been prepared and a drop of the dilute formulation was put on a copper-grid and left to dry. Phosphotungstic acid (2% w/v) was used to stain the sample was dried. Finally, the sample was analyzed by a transmission electron microscope (Arora *et al.,* 2016).

2.8.5 Determination of *in vitro* drug release

These tests were carried out by use of the dialysis bag method to compare the release behavior of the SM-PS phytosome formulation with the suspension formulation (phosphate buffer pH 7.4). The dialysis bags were filled with the optimized formulations. Bags were submerged in phosphate buffer with a pH of 7.4 (25 ml) as a release medium and the medium was kept at an ambient temperature. Using the same quantity of dissolving media, one ml of formulation was obtained at 0, 0.25, 0.5, 1, 2, 4, 6, and 12 h intervals while preserving the sink conditions. To analyze the samples UV spectroscopy at a wavelength of 416 nm was used (Verma *et al.,* 2022).

2.8.6 Determination of kinetic study

The *in vitro* drug release data was analyzed using several mathematical models in a kinetic study (Arora *et al.,* 2016).

2.9 Determination of antiulcer activity

2.9.1 Investigational animals

Adult wistar rats (150-210 g, 12-16 weeks) of either sex were used for the experiment. Animals were housed in polypropylene plastic cages with sawdust as bedding and maintained at standard light, humidity, and room temperature (19-25°C; 12 h light and dark cycle). The animals were acclimatized for one week, during this period animals were fed standard pellets and tap water was provided *ad libitum*. The Investigative procedure accepted by the I.A.E.C. (No. IU/IAEC/21/11) succeeding the rules of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) was adopted.

2.9.2 Grouping and dosing of animals

The antiulcerogenic activity of SM and SM-PS was determined by using six groups containing of six rats each. All the rats were allowed water and destitute of food for 24 h previous to being exposed to ulcerogenic indomethacin using a vehicle (1% CMC) was orally administered except Group I (negative control) and Group VI (Per se). After 24 h, rats were treated with test drugs suspended in 1% CMC. Group I (-ve control) administered 1 ml/kg/day p.o. of 1% CMC only and Group II considered (+ve control) administered 20 mg/kg/day p.o. of indomethacin. Treatment Groups III and IV were treated with SM of 100 mg/kg and SM-PS of 20 mg/kg. Group V was treated with 10 mg/kg ranitidine (Standard drug) and Group VI (per se) was administered SM-PS 20 mg/kg p.o. At the end of the experiment, the animals were sacrificed by an overdose of thiopental sodium (50 mg/kg). The stomachs were removed, and the lesions were macroscopically examined. The diameter of each lesion was measured by planimetry using a simple magnifier (Sabiu *et al.,* 2015).

2.9.3 Quantification of ulceration

The severity of the ulceration was measured in indomethacin-treated animals. The thoroughly cleaned stomachs were fixed to a cork board, and ulcers were graded from 0 to 5 scale (which represents the degree of vascular cramming and wounds/erosions) as shown in (Table 2).

Table 2: Ulcer index description and scores

Ulcer grade	Description
0	Normal mucosa
1	Vascular congestions
2	One or two lesions
3	Severe lesions
4	Very severe lesions
5	Mucosa full of lesion

Regions of mucosal tissue injury were indicated as a percentage of the total surface area of the glandular stomach which was calculated in square millimetres. The proportion of blockage against the ulceration was calculated using the following formulas for the ulcer index (U.I.), which represents the mean ulcer score of all animals (Khushtar *et al.,* 2016).

U.I. = [Ulcerated area/total stomach area] × 100.

%Ulcer inhibition = [U.I. in control – U.I. in test] × 100/U.I. in control

2.9.4 Determination of thiobarbituric acid reactive substances (TBRS)

A capacity of 30% 0.5 ml aqueous solution of trichloroacetic acid (TCA) was poured into 1 ml of 10% tissue homogenate, suspension media containing 0.5 ml of 0.8% aqueous TBA reagent in several test

tubes (Khushtar *et al.,* 2018). The test tube content was protected by aluminum foil and saved in a shaking thermal bath at 80 ± 4°C for 30 min. The test tube was placed in ice cold water for 30 min and centrifuged at 2500 rpm for 20 min. The absorbance of the supernatant was taken at 540 nm in contradiction of the blank reagent.

2.9.5 Assessment of catalase action

The stomach muscle homogenate was homogenized by using 50 mM phosphate buffer in a 1:12 ratio. The content was then centrifugated at 85,00 rpm at 5°C for 15 min. An aliquot of 60 µl supernatant was poured to a cuvette holding 2.95 ml of 20 mM hydrogen peroxide (H_2O_2). The variation in the absorbance (Abs/min) was noted at one-minute intervals for the 3 min at λ_{max} 250 nm. The incidence of catalase smashes H_2O_2 leading to a diminution in the absorbance (Khushtar *et al.,* 2018). The action of catalase was intended using the formula:

n moles of H_2O_2 used up/min/mg protein = (Abs/min × capacity of assay)/(0.081 × capacity of homogenate × mg of protein).

2.9.6 Effect of SM and SM-PS on superoxide dismutase (SOD) activity

This activity is based on that the superoxide anion radical catalyzes the autooxidation of pyrogallol. An aliquot of 80 µl of supernatant of stomach muscle homogenate was poured into the tris-HCl buffer and made up the volume of 3 ml with the buffer solution (A). Solution (B) was prepared by dissolving 20 µl (20 mM) pyrogallol in 10 mM HCl and poured in solution (A). The variations in absorbance at $ë_{max}$ 425 nm were noted at 1 min intervals for 3 min. The intensification in absorbance after adding the pyrogallol was subdued by the existence of SOD (Khan *et al.,* 2019). One part SOD was designated as the quantity of the enzyme essential to trigger 50% embarrassment pyrogallol by 3 ml autooxidation of assay assortment and was intended by the formula:

Parts SOD in 1 ml of mixture = (ΔC – ΔT) × 100/ΔC × 50

where ΔC is absorbance alteration per min. in control, ΔT is the change of absorbance in per min. in the tested mixture. All the data was articulated as parts of SOD in 1 mg of protein suspension.

3. Results

3.1 Analysis of bioactive compounds

The purpose of the GC-MS analysis was to find out the biologically active compounds present in the SM. The outcome shows that there are 37 compounds identified, in which phenolic compounds and steroids are the major components. Figure 1 shows the GC-MS chromatograms and shows the major active components, peak area, concentration, and retention period. The major existing compounds identified in SM were phenolic compounds like brenzkatechin (0.40%), pyrogallol (88.35%), diethyl phthalate (3.66%), and steroids like gamma sitosterol (0.91). The GC-MS data could be encouraging for the field of pharmaceutical research sector in the detection of different phytoconstituents in SM (Figure 1).

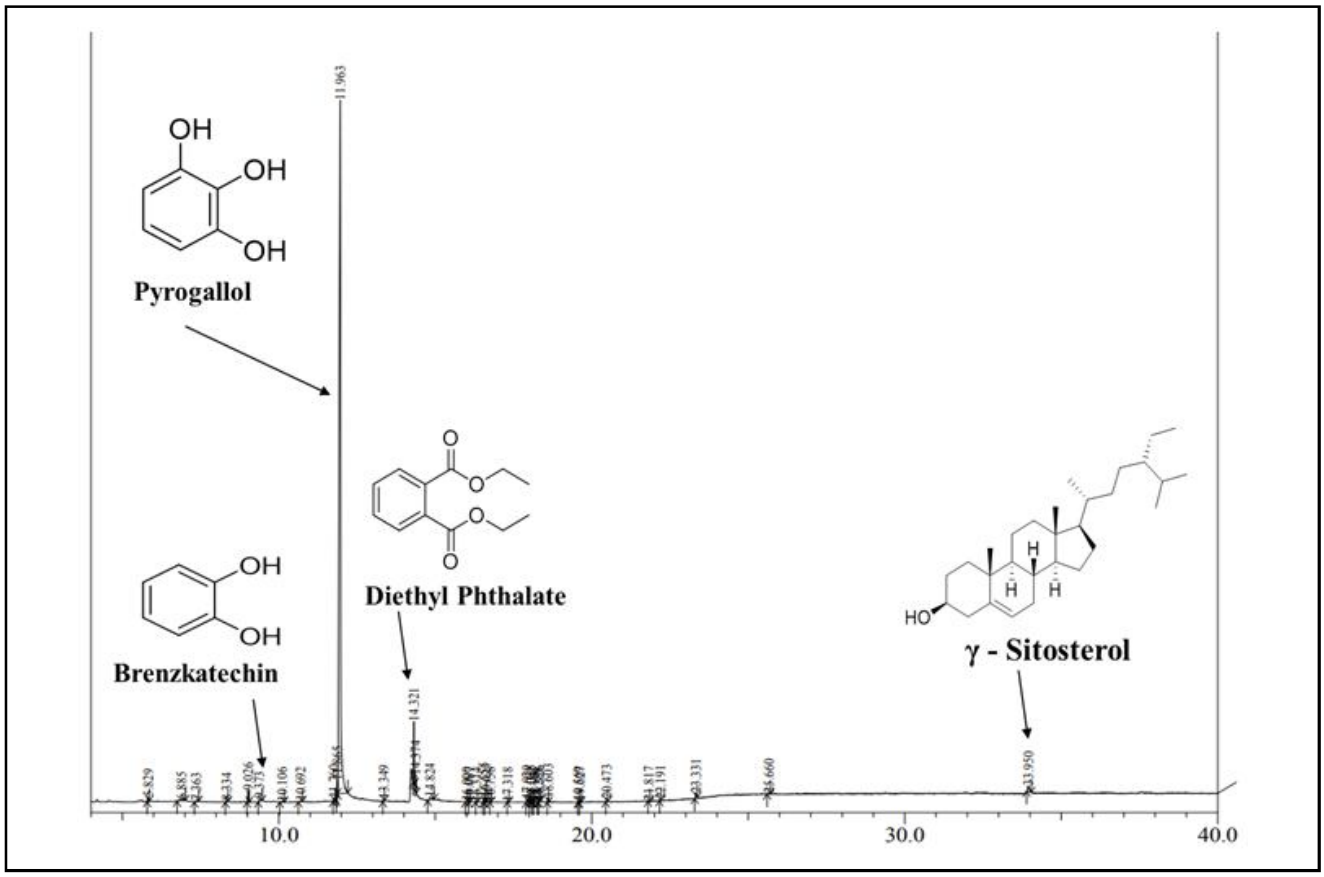


Figure 1: GC-MS Chromatogram of methanol extract of *S. mangifera* (SM).

3.2 Construction and optimization of SM-PS by Box-Behnken design

For the development of SM-PS, the BBD generated 17 runs. A quadratic model was determined to have the best fit for responses in all the preparations. The R^2 values, S.D., and %CV of all three variables is revealed in (Table 3).

3.2.1 Response 1 (Y_1): Effect of independent variables on vesicles size

As the amount of lipid increased from 40 to 60, the corresponding size also increased from 79.5 nm (Formulation 14) to 180.6 nm (Formulation 16). The effect is demonstrated in 3D response plots as shown in Figure 2 (A-C).

3.2.2 Response 2 (Y_2): Outcome of independent variables on PDI

PDI of all phytosomes formulations ranged from 0.143 to 0.433, with an average PDI of 0.274. As per equation Y_2 shown in Table 3, lipid and cholesterol were found to have a positive fallout on PDI whereas sonication time has a negative fallout, but this is not significant. With an increase in lipid concentration, the PDI of the SM-PS phytosome increased from 0.146 (formulation 14) to 0.433 (formulation 16). The fallout is seen in Figure 2 (D-F).

Table 3: Summary of results of regression analysis for responses Y_1, Y_2 and Y_3 for fitting to the quadratic model

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Mean	SD	%CV	Adequate precision
Response (Y_1)	0.9970	0.9931	0.9525	124.02	2.64	2.13	52.4567
Response (Y_2)	0.9915	0.9805	0.8717	0.2741	0.0130	4.73	31.8936
Response (Y_3)	0.9892	0.9752	0.8319	81.99	1.31	1.60	28.8612

$Y_1 = + 117.50 + 42.56A + 10.61B - 4.45C - 0.8750AB + 0.8500AC - 2.75BC + 11.46A^2 + 1.96B^2 + 0.4375C^2$

$Y_2 = + 0.2446 + 0.1209A + 0.0378B - 0.0051C - 0.0013AB + 0.0005AC - 0.0013BC + 0.0389A^2 + 0.0057B^2 + 0.0180C^2$

$Y_3 = + 84.46 + 10.83A + 3.45B + 0.0250C - 1.58AB - 0.7750AC + 0.6750BC - 2.77A^2 - 2.07B^2 - 0.4175C^2$

3.3.3 Response 3 (Y_3): Outcome of independent variables on entrapment efficiency

The average entrapment efficiency (EE) of each SM-PS formulation was 81.99%, with a minimum and highest value of 62.7% and 93.4%, respectively. The concentration of lipids was shown to have an affirmative result on EE; however, the other two variables, cholesterol, and sonication time were obtained to have an adverse effect on EE. The overall effect of sonication time on %EE was found to be positive as seen in Figure 2 (G-I).

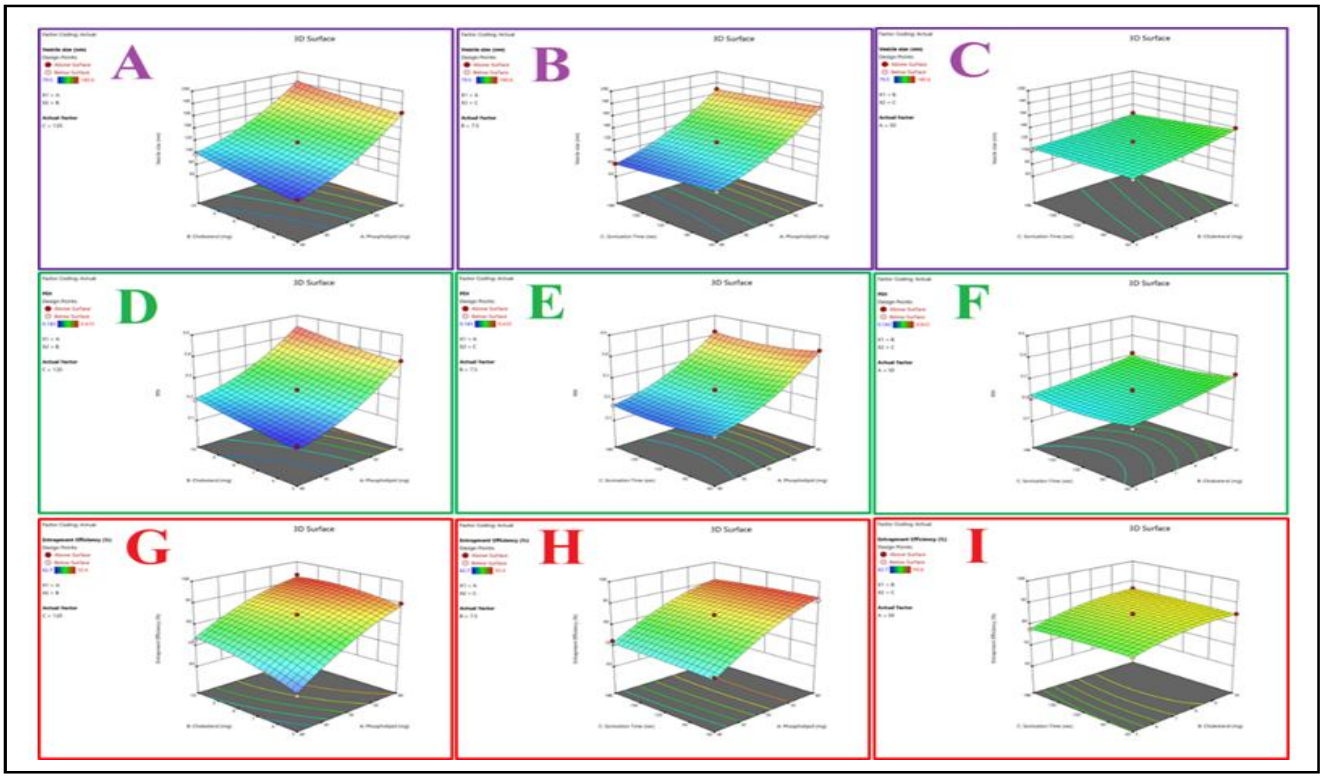


Figure 2: 3D-response surface plot showing the effect of independent variables on vesicle size (A-C) polydispersity index (D-F) and entrapment efficiency (G-I).

3.4 Characterization of SM-loaded phytosome

3.4.1 Vesicle size and polydispersity index (PDI)

The vesicle size of SM-loaded phytosomes was found to be 117.4 nm and its PDI was 0.247 (Table 1).

3.4.2 Zeta potential of SM-loaded phytosomes formulation

The zeta potential of SM-loaded phytosome was found to be -24.24 mV as shown in (Figure 3).

3.4.3 Entrapment efficiency

The average percentage entrapment efficiency of SM-PS was found to be 81.99% (Table 3).

3.4.4 Transmission electron microscopy (TEM)

The developed vehicles were well-defined sealed structures with homogeneous size distribution and spherical in shape as shown in (Figure 3).

Table 4: Selected independent and dependent variables for the preparation of SM-phytosome by Box-Behnken design

Variables	Low (-1)	Medium (0)	High (+1)
Independent variables			
X_1 = phospholipid (mg)	40	50	65
X_2 = Cholesterol (mg)	5	7.5	10
X_3 = Sonication time (second)	60	120	80
Dependent variables			
Vesicle size (Y_1)	Minimize		
Polydispersity index (PDI) (Y_2)	Minimize		
Entrapment efficiency (EE) (Y_3)	Maximize		

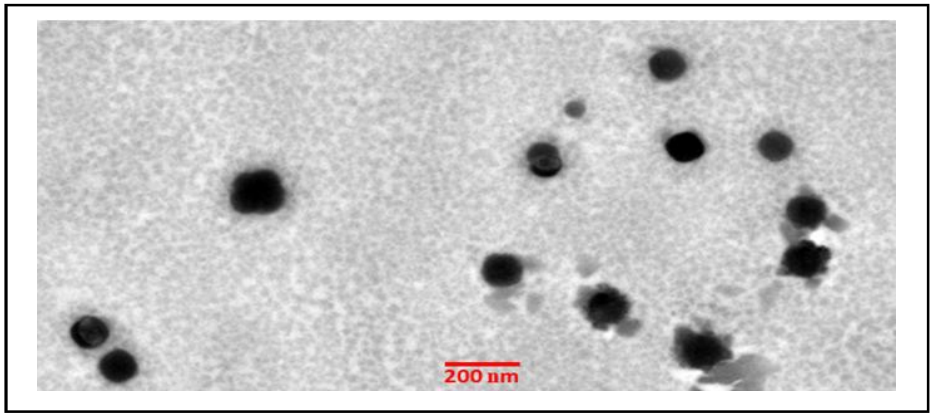


Figure 3: Transmission electron micrograph (TEM) of OPT-SM-PS formulation.

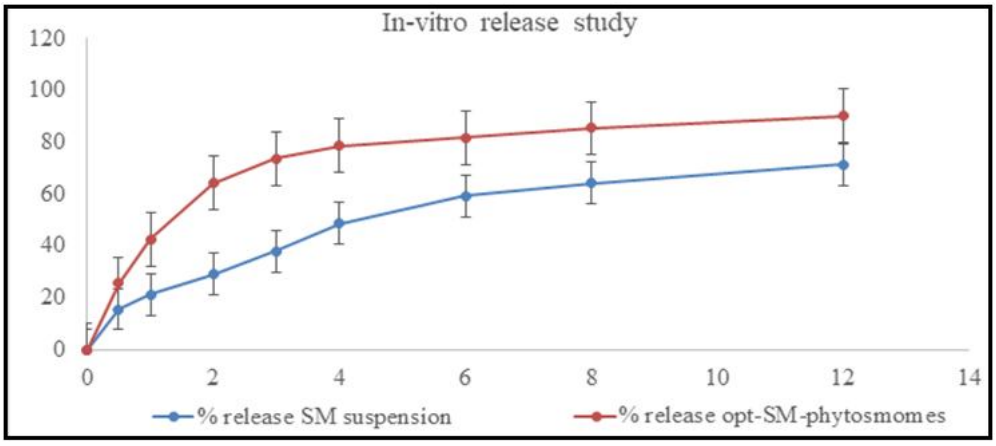


Figure 4: *In vitro* release of a MS drug suspension and the SM loaded phytosome and the *in vitro* release models of the prepared MS-PS.

Table 5: Effects of methanolic extract of *S. mangifera* (SM) and its formulations SM-PS on different ulcer specific variables

Treatment groups and ulcerspecific variables	Ulcer index scores	TBARS (nmol/mg protein)	Catalase (nmol of H_2O_2 consumed/ min /mg protein)	SOD (units/mg protein)
I (Normal Control) 1% CMC: 1 ml/kg	0.00 ± 0.00	1.46 ± 0.17	0.068 ± 0.013	0.72 ± 0.12
II (Ulcer Control) 1% CMC: 1 ml/kg + IND: 20 mg/kg	3.60 ± 0.14**	4.73 ± 0.26**	0.018 ± 0.008**	0.14 ± 0.08**
III (Extract treated) SM 100 mg/kg + IND: 20 mg/kg	1.58 ± 0.62$^{\#}$	3.12 ± 0.28$^{\#}$	0.032 ± 0.014$^{\#}$	0.37 ± 0.12$^{\#}$
IV (Formulation treated) SM-PS 20 mg/kg + IND: 20 mg/kg	1.24 ± 0.23$^{\#\#}$	2.24 ± 0.29$^{\#}$	0.052 ± 0.023$^{\#}$	0.51 ± 0.12$^{\#}$
V (Standard drug treated) Ranitidine: 10 mg/kg + IND: 20 mg/kg	0.97 ± 0.14$^{\#\#}$	1.84 ± 0.18$^{\#}$	0.059 ± 0.014$^{\#}$	0.68 ± 0.11$^{\#}$
VI (Per se) SM-PS 20 mg/kg	0.12 ± 0.26	1.50 ± 0.32	0.064 ± 0.018	0.68 ± 0.09

Each value is expressed in Mean SEM one-way ANOVA ($n=6$) followed by Dunnett's test.
p = $^{*}p<0.05$; $^{**}p<0.01$ ulcer control Group II compared to normal control Group I
p = $^{\#}p<0.05$; $^{\#\#}p<0.01$ tested Group III, IV, and V compared to ulcer control Group II

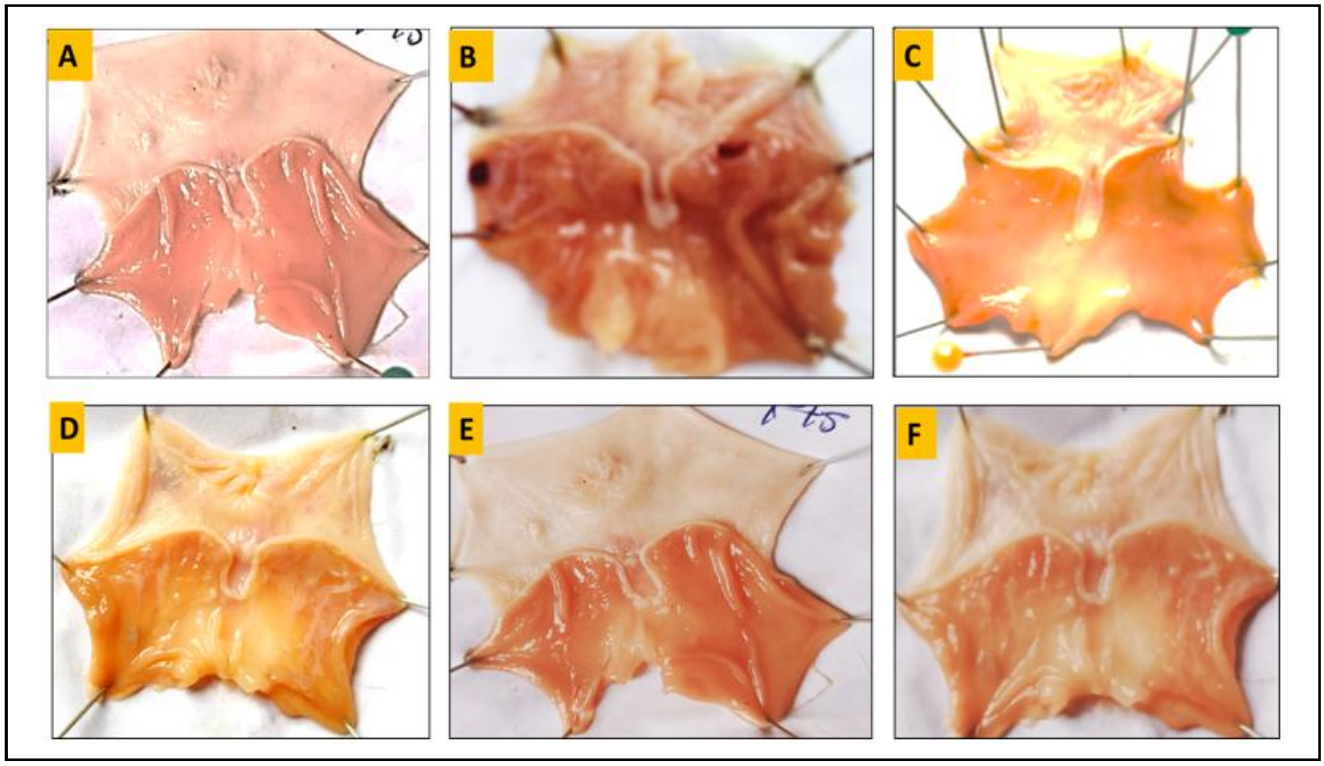


Figure 5: Effect of tested samples of *S. mangifera* bark on the gross structure of stomach [A]. Normal control Group I [B], ulcer control Group II [C], SM treated 100 mg/kg Group III [D], SM-PS treated 20 mg/kg Group IV [E], standard drug treated Group V [F] and per se Group VI.

3.4.5 *In vitro* drug release

The release behaviors of the synthesized SM-PS and suspension SM were evaluated to determine the release of SM. In the release study, SM-PS exhibited a higher drug release (89.14% at 12 h) than SM. At each point in time, a substantial drug liberation was accomplished. While in resemblance to pure SM, the SM-PS showed delayed drug liberation (Figure 4).

3.5 Antiulcer activity

The effects of SM and SM-PS on ulcer index, TBA reactive substance (TBARS), catalase, and SOD levels are shown in (Table 5). In comparison to the Group I, Group II animals showed a significant intensification in UI and TBARS ($p>0.01$) and a significant decline in catalase and SOD ($p<0.01$). Group III, IV, and V rats presented a reduction in UI ($p<0.05$) and TBARS levels, and an increase in catalase and SOD levels ($p>0.05$) related to Group II. Group VI (Per se) animals presented non-significant changes in the UI, TBARS, catalase, and SOD levels in comparison to control Group I.

4. Discussion

The 3-dimensional graph represents the responses of independent factors on vesicular size, %EE, and PDI. The experimental values were quantitatively matched with the predicted values. The average

particle size of each 17 formulation was established to be 124.02 nm with the values ranging from 79.5 nm to 180.6 nm. Equation Y_1 in (Table 1) shows an increase in vesicle size with the increase in lipid amount, while cholesterol had a negative effect. Formulation containing the highest amount of lipid (60 mg) exhibited %EE of 93.4% while the one with the lowest lipid content (40 mg) showed %EE of 62.7%. This effect can be seen in Figure 2 (G-H). A relationship was observed between cholesterol and %EE. Increase in cholesterol from 5% to 10% increase %EE from 62.7%. (Formulation 14) to 93.4% (Formulation 16). The higher value of zeta potential is because of the anionic nature of cholesterol which gets adsorbed on the particle/water interface and forms an electric double layer which leads to higher stability. PS can edge drug liberation because SM must traverse the lipid bilayer and is capable of diffusing gradually. From Figure 4, formulation liberated quickly in the first and two h, and afterward at a lesser degree over the next 12 h. This kind of liberating performance is ideal for enriching the useful treatment. Preliminary fast liberation of the drug helps in attaining therapeutic strength, but prolonged slow release expands therapeutic efficacy.

A high dose of indomethacin leads to erosion of the mucosal layer and is liable for the induction of ulceration (Andrews *et al.*, 1994). Hence, indomethacin was utilized in the experiment to tempt ulcers in animals. Outcomes of research verified that Group II rats exhibited an intensification in UI and TBARS whereas a diminution in catalase and SOD levels was related to normal control Group I. Outcomes of the study confirmed that pretreatment with SM, SM-PS, and ranitidine (Group III, IV, and V) significantly minimizes the ulceration effect of indomethacin and maintains the TBARS, catalase, and SOD levels in comparison to Group II. In the present study, reactive oxygen metabolite (TBARS) was estimated because large quantities of TBRS formation have been concerned with the pathogenesis of several inflammatory situations including gastrointestinal tract ailments and peptic ulcers (Vijay *et al.,* 2022). ROS construction increases defensive willpower in coming back to certain external stimuli, unfavourable diets, and human ailments. Generally, these ROS are counterbalanced by endogenic antioxidant cellular systems such as catalase and SOD. Through oxidative stress, a situation will happen when ROS formed is gathered and overdoes the cellular antioxidant system. Therefore, oxidative stress is responsible for the initiation and magnification of gastric ulcers. The endogenic antioxidant cellular system catalase and SOD are important for the body in command to release injurious ROS from the cellular system. These findings showed that catalase and SOD levels were significantly increased in SM, SM-PS, and ranitidine (Group III, IV, and V) in comparison to the ulcer control Group II animals where it decreased. These outcomes recommended the conceivable participation of endogenous antioxidants in the experimental consequence of SM and SM-PS in gastric ulcers. The GC-MS analysis of SM exhibits the presence of phenolic compounds brenzkatechin (0.40%), pyrogallol (88.35%), and diethyl phthalate (3.66%), which have strong antioxidant and antiulcer activity. These compounds help control the generation of TBRS in stress conditions (Wahab *et al.,* 2014).

5. Conclusion

The phytoconstituents present in the methanolic extract of *S. mangifera* were qualitatively analyzed by GC-MS. The analysis disclosed the presence of diverse medicinally active phenolic compounds and steroid compounds. As per the literature report, these are the active antiulcer compounds. The present verdicts conclude that the methanolic extract of *S. mangifera* bark and formulated phytosomes (SM-PS) have antiulcerogenic activity as they have exhibited a protective effect on gastric ulcers in rat models. The outcome of the research work also proposes that the optimized phytosome based drug delivery system containing herbal extracts as bioenhancers has the potential to improve the bioavailability of hydrophilic and hydrophobic components of extracts. We anticipate that the inhibition of gastric damage by formulated phytosomes (SM-PS) is more effective than bark extract at low doses due to the improved bioavailability of phenolic compounds present in the extract. Further studies shall emphasize on the isolation of phenolic compounds, formulation development, and elucidating mechanisms of action.

Acknowledgments

The researchers are grateful to the research and development committee Integral University, Lucknow to perceive the work from time to time and stipulating the manuscript submission number (IU/R&D/2024-MCN0002399).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Reference

Ajay, S.; Amit, K. and Rupesh (2023). Nanoemulsion: A futuristic approach to disease elimination for food safety. Ann. Phytomed., **12**(1):180-186.

Andrews, F.J.; Malcontenti-Wilson, C. and O'brien, P.E. (1994). Effect of nonsteroidal anti-inflammatory drugs on LFA-1 and ICAM-1 expression in gastric mucosa. Am. J. of Physiol-Gastrol and Liver Physiol, **266**(4): G657-G664.

Arif, M.; Zaman, K.; Fareed, S. and Hussain, M. (2008). Antibacterial, antidiarrhoeal and ulcer-protective activity of methanolic extract of *Spondias mangifera* bark. I. J. H. R, **2**(1): e211, 3-8

Arora, D.; Khurana, B.; Narang, R. K. and Nanda, S. (2016). Quality by design (QbD) approach for optimization and development of nano drug delivery systems. Trends Drug Deliv, **3**:23-32.

Brooks, F.P. (1985). The pathophysiology of peptic ulcer disease. Digestive Diseases and Sci, **30**:15S-29S.

Khan, A.; Khan, S.; Khan, M.M.; Alam, R. and Srivastava, V.K. (2019). Comparative study of malondialdehyde and superoxide dismutase in diagnosed COPD patients. Indian J. Basic and Appl. Medical Res, **8**(2):189-196.

Khan, A.B.; Arif, M.; Ahmad, U.; Kushwaha, S.P.; Alyahya, S. and Shafi, S. (2022). *Spondias mangifera* Willd.: A highly healthful turpentine aromatic plant for the management of the seasonal affective disorders. Ann. Phytomed, **11**(1):221-235.

Khushtar, M.; Ahmad, A. and Rahman, M.A. (2018). Gastroprotective effect of hydro-alcoholic extract of *Polygonum bistorta* Linn. root in indomethacin-induced gastric ulcers in sprague dawley rats. Indian J. of Pharmaceutical Edu. and Res, **52**(4):618-625.

Khushtar, M.; Siddiqui, H.H.; Dixit, R.K.; Khan, M.S.; Iqbal, D. and Rahman M.A. (2016). Amelioration of gastric ulcers using a hydro-alcoholic extract of Triphala in indomethacin-induced Wistar rats. European J. Integr. Med., **8**(4):546-551.

Kumar, S.; Arif, M.; Jawaid, T.; Al-Khamees, O.A.; Anjum, A. and Shafi, S.; Thirunavukkarasu, V.; Josephine, S.P.; Muteeb, G.; Singh, K. and Qadir, A. (2023). Antiplatelet and thrombolytic activity of phenolic-insistent fractions from the new-fangled stem of *Ficus benghalensis* with concurrent GC-MS analysis. Intelligent Pharmacy, **1**(4):224-231.

Muhammad, A.; Zaman, K. and Sheeba, F. (2009). Pharmacognostical studies and evaluation of total phenolic contents of trunk bark of *Spondias mangifera* Willd. Natural Product Radiance, **8**(2):146-150.

Qadir, A.; Ali, A.; Arif, M.; Al-Rohaimi, A.H.; Singh, S.P.; Ahmad, U. and Kumar, A. (2018). Solvent extraction and GC-MS analysis of sesame seeds for determination of bioactive antioxidant fatty acid/fatty oil components. Drug Research, **68**(06):344-348.

Qadir, A.; Aqil, M.; Ahmad, U.; Khan, N.; Warsi, M.H. and Akhtar J. (2020). Date seed extract-loaded oil-in-water nanoemulsion: Development, characterization, and antioxidant activity as a delivery model for rheumatoid arthritis. J. Pharm. Bioall. Sci, **12**:308-316.

Sabiu, S.; Garuba, T.; Sunmonu, T.; Ajani, E.; Sulyman, A.; Nurain, I. and Balogun, A. (2015). Indomethacin-induced gastric ulceration in rats: Protective roles of *Spondias mombin* and *Ficus exasperata*. Toxicol. Reports, **2**:261-267.

Sandhya, R.; Mandadi and Lankalapalli, S. (2023). Synthesis and characterization of letrozole-loaded polymer based nanoparticulate formulations for treating breast cancer. Ann. Phytomed, **12**(1):310-317.

Sharma, S.; Hafeez, A. and Usmani, S.A. (2022). Nanoformulation approaches of naringenin: An updated review on leveraging pharmaceutical and preclinical attributes from the bioactive. J. of Drug Delivery Sci. and Tech., pp:103-724.

Singh, B.; Dahiya, M.; Saharan, V. and Ahuja, N. (2005). Optimizing drug delivery systems using systematic" design of experiments." Part II: retrospect and prospects. Critical Reviews in Therapeutic Drug Carrier Systems, **22**(3):27-106.

Singh, B.; Kapil, R.; Nandi, M. and Ahuja, N. (2011). Developing oral drug delivery systems using formulation by design: Vital precepts, retrospect, and prospects. Expert Opin. Drug Deliv, 8:1341-1360.

Singh, K.; Singh, L.R. and Mishra, A. (2022). Therapeutic potential of indigenous medicinal plants against gastric ulcer. Ann. Phytomed., **11**(1):206-214.

Usmani, A.; Mishra, A.; Jafri, A.; Arshad, M. and Siddiqui, M.A. (2019). Green synthesis of silver nanocomposites of *Nigella sativa* seeds extracts for hepatocellular carcinoma. Curr. Nanomaterials, **4**(3):191-200.

Verma, M.; Fatima, S. and Ansari, I.A. (2022). Phytofabricated nanoparticle formulation for cancer treatment: A comprehensive review. Curr. Drug Metabol., **23**(10):818-826.

Vijay, L.; Karan, C.; Chaudhari, Vipul. G. and Surendra, A. (2022). Protective effects of *Fagonia cretica* L. aqueous extract on experimentally induced gastric and duodenal ulcers in wistar rats. Ann. Phytomed, **11**(2):421-425.

Wahab, S.; Hussain, A.; Farooqui, A.H.A.; Ahmad, M.P.; Hussain, M.S.; Rizvi, A.; Ahmad, M.F. and Ansari, N.H. (2014). *In vivo* antioxidant and immunomodulatory activity of *Bombax ceiba* bark: Focusing on its invigorating effects. Am. J. Adv. Drug Deliv, **2**(1):1-13.

Citation **Abdul Baseer Khan, Muhammad Arif, Usama Ahmad, Syed Misbahul Hasan, and Mohd. Khushtar (2024). GC-MS analysis and formulation of novel antiulcer phytosomes from methanol extract of *Spondias mangifera* Willd. stem bark. Ann. Phytomed., 13(1):667-675. http://dx.doi.org/10.54085/ap.2024.13.1.68.**