Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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Research Article

Formulation and Evaluation of Transdermal Patch Containing Withania Coagulans

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A B S T R A C T

The Withaniacoagulans L. Dunal, a small bush, belongs to the family Solanaceae and is widely spread in India, Pakistan, Afghanistan and South Asia. It is commonly known as 'Indian cheese maker' or 'vegetable rennet' due to its milk coagulating property found especially in its leaves and fruits. In the present study phytochemical screening of methanolic fruit extract of Withaniacoagulans was performed. Fourier Transform Infrared Spectroscopy analysis was performed for screening Withanolides, having potential for treatment of diabetes in natural way. Different withanolides are responsible for different therapeutic properties. Transdermal drug delivery systems have gained significant attention as an effective Alternative to conventional oral administration for Various therapeutic agents. In this study, we aimed To develop and evaluate transdermal patches ofwithaniacoagulans.

KEYWORDS: Indian cheese maker, Transdermal drug delivery system, Transdermal patch, Fourier Transform infrared spectroscopy.

A R T I C L E I N F O: Received 14 Jan 2024; Review Complete 16 March 2024; Accepted 23 May 2024; Available online 15 June. 2024

Cite this article as:
Manikpuriya S, Gandakhe I, Shinde N, Pawar S, Sanap G, Formulation and Evaluation of Transdermal Patch Containing Withania Coagulans, Asian Journal of Pharmaceutical Research and Development. 2024; 12(3): 66-74, DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1396

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INTRODUCTION:

Transdermal drug delivery systems are defined-surface adhesive drug delivery devices that apply a preset dosage of medication at a pre-programmed pace to the surface of intact skin. In order to maintain acceptable plasma drug levels for therapeutic efficacy, these systems input the drug at the proper rates.[1]Due to its potential to avoid hepatic first pass metabolism and achieve high systemic bioavailability for drugs that undergo either significant or extensive first pass metabolism and are capable of maintaining drug release for an extended period of time, transdermal delivery of drugs for systemic treatment of diseases has attracted increasing interest in recent years. Additionally, it offers quick termination of necessary medication and suitability for self-administration, improving patient compliance.[2]Despite these benefits, only a small number of medications can be used topically because most medications have minimal skin permeability. It has been established that the stratum corneum is a highly effective barrier against skin penetration. Vehicles, penetration enhancers like DMSO (dimethyl sulfoxide), pyrrolidone, urea, and electrotransport will be employed to get around these issues. Many medications have been tried to be delivered transdermally using facilitated transdermal systems.[3] Fundamentals of transdermal penetration Among other things, a medicine applied topically needs to pass through the skin's Statumcorneum, or penetration barrier. Three folliculle regions and sweat glands allow drug molecules to permeate the skin.[4]Dry molecules may first transiently penetrate the epidermis through sweat ducts or hair follicles, and then then enter the body through the sebaceous glands and follicular epithelium. Diffusion via the Statumcorneum becomes the main route after a Steady state is reached.

Figure 1: Plant of withaniacoagulans

Withaniacoagulans Paneer in Tamil and Rishyagandha in Sanskrit Known by its Hindi name, PaneerPhool Pashto.[5] this plant belongs to the nightshade or solanaceae family and is indigenous to Afghanistan, Pakistan, and the Indian subcontinent.[6] Two economically significant withania species are w. somnifera (Ashwagandha) and w. coagulans (Paneerdoddi/Ashutoshbooti), which are grown throughout various locations for their Ayurvedic use.[7] It is supposed to aid with diabetic management.[8]The berries have a protease that resembles rennet that can be used to coagulate milk in order to make cheese.[9, 10]Alternariaaltarnata, a leaf spot disease, is prone to affect the plant.[11]

Since ancient times, people have utilized plants as traditional healing methods and as possible sources of medicinal chemicals. Certain therapeutic plants are rich in a variety of bioactive components. It has been observed that these bioactive components can prevent and treat a variety of illnesses to help people live healthy lives. Because of its potent pharmacological and nutraceutical qualities, Withaniacoaguians is one of the most significant medicinal plants in the genus Withania in the Ayurvedic medical system. It is grown all across the world, from North Africa to South Asia and the Mediterranean region.[12] Just two of the twenty-three species of the genus Withania that have been identified—W. coagulans and W. somnifera—have any commercial significance.[13]Because of an enzyme found in its berries, this plant—also referred to as an Indian cheesemaker—is frequently used to coagulate milk.[14] shows the W. coagulans plant's leaves, stalks, and fruit. The fruit, leaves, and roots are all medicinally beneficial. Berries are mostly composed of alkaloids, amino acids, esterases, and essential oils.[15] The plant's therapeutic qualities are ascribed to "Withanolides," which are steroid derivative chemicals.The entire plant contains a number of withanolides, including coagulin F, coagulanolide, withacoagulin, and coagulin G.

The plant's ripe fruit has a sweet taste and is used as a sedative, to treat dyspepsia, asthma, and wound healing. As an antibacterial,[17] antimicrobial,[18]hepatoprotective,[19]hypolipidemic,[20] antioxidant,[21] anti-tumor,[22] anti-depressant,[23] immunosuppressive,[24] and anti-inflammatory agent,[25] dry fruit is also traditionally used as a treatment for diabetes in many countries. While flower buds demonstrated anthelmintic activity, seeds are beneficial for lowering inflammation, acting as a diuretic, and treating ophthalmia.[26,27, 28] In parts of South Asia, the plant's twigs are utilized as blood purifiers, pain remedies, and teeth cleaners. Taking into account the increasing use of This review aims to provide thorough knowledge on phytochemistry, dietary usage, and the therapeutic potential of w.coagulans, as well as medicinal plants and their application in diverse indigenous health systems.

EXPERIMENATAL WORK:

MATERIAL:

Collection of plant material:

Figure 2: Withania Coagulans fruit and powder

The medicinal plant (fruits) of withaniacoagulans (solanaceae) used in the study were collected from local market of ChhatrapatiSambhajinagar, Maharashtra.Collected fruits were authenticated from botanical survey of Dr. Babasaheb Ambedkar Technological University, Maharashtra.

Cultivation:

W. coagulans, also referred to as "PaneerDoda" or Indian Rennet, is mostly grown in areas where it is native or has evolved to grow. India is one of the primary cultivation regions for Withaniacoagulans, which is widely grown throughout the country. Particularly, Rajasthan, Gujarat, Punjab, and Haryana's arid and semi-arid regions are where it is farmed. These areas offer the right soil and climate conditions for its growth.[29]

EXTRACTION:

Figure 3: Extraction of withaniacoagulans

The methanolic extract of withaniacoagulans was made by continuous soxhlet extraction with 50% methanol for 24 hrs. The methanolic substance was evaporated to obtain the thick extract.

Phytochemical studies:

Detection of Alkaloids

Dilute Hydrochloric acid was added to the extracts and then the solution was filtered.

a. **Mayer's test:** The filtrate was treated with Potassium Mercuric Iodide (Mayer's reagent). The formation of a yellow coloured precipitate indicates the presence of alkaloids in the extracts.
b. **Hager's test:** The filtrate was treated with saturated picric acid solution (Hager's reagent). Yellow colour precipitate formation confirmed the presence of alkaloids.
c. **Dragendroff's test:** The filtrate was treated with the solution of Potassium Bismuth Iodide known as the Dragendroff's reagent. A formation of a red precipitate will indicate the presence of alkaloids.
d. **Wagner's test:** To 1 ml of the extract, added 2 ml of Wagner's reagent. The formation of a reddish brown precipitate indicated the presence of alkaloids.

Detection of carbohydrayes:

The extracts were dissolved in distilled water (5ml) and then the filtrates were used for carrying out the tests.

a. **Molish test:** 2 drops of alcoholic α-naphthol solution was added to the filtrate. The presence of carbohydrates is indicated by the formation of a violet ring at the junction.
b. **Fehling test:** 1 ml of Fehling's solution A and 1 ml of Fehling's solution B were added to five drops of the test filtrates. Presence of reducing sugars is indicated by the presence of red precipitate.
c. **Benedict test:** Five drops of filtrate was added to 2 ml of Benedict's reagent and then the solution was heated in water bath for few minutes. The formation of orange/red precipitate indicates the presence of reducing sugars.

Detection of glycosides

a. **Borntrager's test:** : 0.5 g of the plant extract was shaken with benzene and organic layer got separated and half of its own volume of 10% ammonia solution added. A pink, red or violet colouration indicated the presence of glycosides.
b. **Baljet test:** To 1 ml of the test extract added 1 ml sodium picrate solution and the yellow to orange color revealed the presence of glycosides.

Detection of Phytosterol

a. **Salkowskis test:** The extract was treated with chloroform and filtered. The filtrate was then treated with a few drops of concentrated Sulphuric acid and the solution was

shaken and allowed to stand for a few minutes. The appearance of yellow golden colour indicates the presence of triterpenes.

b. Lieberman's Burchard test: Dissolved the extract in 2 ml of chloroform in a dry test tube. Added 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The solution became red, then blue and finally bluish green, indicated the presence of steroids.

c. Ninhydrin test: Added two drops of freshly prepared 0.2% ninhydrin reagent to the extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

Detection of saponins

Foam test: 0.5ml extract was taken and mixed with 2ml of water. The solution was shaken for few minutes. The presence of saponinsis indicated if the foam is persists for more than 10 minutes.

Detection of flavonoids and protein

Lead acetate test: To the extract, 1 ml ofLead acetate solution was added. Formation, of a white precipitate indicated the presence of proteins or flavonoids.

Figure 4: Observations of test

Table 1: Phytochemical screening of withaniacoagulans extract:

Sr. No	Chemical constituente	Test	Observations
1.	Alkaloids	Mayers test	+
		Hagers test	+
		Dragendorffs test	+
		Wagners test	+
2.	Carbohydrates	Molish test	–
		Fehlings test	–
		Benedict test	+
3.	Glycosides	Bomtrager test	+
		Balietatest	+
		Foam test	+
4.	Phytosterol	Salkowaskis test	–
		Liebermann Burchardtest	+
		Ninhydrin test	–
5.	Flavonoids	Lead acetate test	+
6.	Proteins	Lead acetate test	+

Procedure of transdermal patch:

General method of preparation: In a present study drug in adhesive type trandermal patches of withaniacoagulans were prepared by solvent casting method.

Table 2: Concentration of ingredients for formulation

w.coagulans extract	0.256ml/4cm^2 area of patch
Hydroxy propyl methyl cellulose	0.100gm
Poly vinyl pyrrolidone	0.100gm
Dimethyl sulfoxide	3ml
Propylene glycol	4ml

Solution 1: Required amount of water (ml) was taken into a beaker and was heated at about 50-60°C.The stated quantities of HPMC and PVP were dissolved in the about water until completely dispersed and a gel like consistency is obtained.

Solution 2: An accurately weighed quantity of drug (0.0256ml) was dissolved in a required quantity of ethanol DMSO and propylene glycol was added.

Solution 1 was added to the solution 2 with continuous stirring by using magnetic stirrer.

The polymeri drug dispersion was then poured into the petri plate containing performed backing layer of aluminum foil.the solution was covered with an inverted glass funnel to allow controlled evaporation of the solvent and to avoid blistering effect of the film for 24hrs at room tempreture.

Figure 5: Preparation of transdermal patch

RESULT AND DISCUSSION:

Evaluation parameters:

Physical Appearance:

All the transdermal patches were visually inspected for colour. Flexibility, homogeneous and smoothness. The films are found to have uniformly creamy white coloured with homogeneous appearance, flexible and smooth surface texture.

Film Thickness: The thickness was measured at five different places using a digital micrometer and the mean values were calculated

Weight Uniformity:

Three film were weigh individually and average weight was calculated

Folding Endurance:

The folding endurance is expressed as the number of folds (number of times the film is folded at the same place) required to break the specimen or to develop visible cracks. This gives an indication of brittleness of the film. A small strip of film (2cm x 2cm) was subjected to this test by folding the patch at the same place repeatedly several times until a visible crack was observed.

Moisture Content:

Moisture content can influence the mechanical strength and drug release behavior of the transdermal therapeutic systems and therefore, in the present study determination of the moisture of the formulated patch was estimated by keeping the patch under vacuum desiccation until constant weights were obtained. The percentage moisture content of the patch was calculated by the formula.

% of moisture content (initial weight-final weight) initial weight ×100

% of Moisture Uptake:

The patch was weighed accurately and placed in desiccators containing aluminum chloride. After 24 h, the patch was taken out and weighed. The percentage moisture uptake was calculated as the difference between final and mitial weight. It is calculated by using following formula.

% of moisture uptake (final weight initial weight)/initial weight ×100

% Drug Content:

Small pieces of patches were cut into fragments and submerged in a phosphate buffer solution with a pH of 7.4 for duration of 24 hours Subsequently, the entire solutionunderwentultrasonication for 15 minutes. After filtration, the amount of drug present was quantified spectrophotometrically at the wavelength of maximun absorption, 230 nm

Percentage Elongation:

When a patch is subjected to stress, it undergoes stretching, which is referred to as strain Strain, fundamentally, is the deformation of the patch divided by its original dimensions. Typically, the elongation of a patch increases with higher plasticizer content. It is calculated by using following formula

Percentage of elongation increase in length of the patch/ initial length of the patch ×100

Determination of Surface pH:

The patches underwent a swelling process by immersion in 1 ml of distilled water for 2Hours at room temperature. The pH of the solution was measured using an electrodePlaced on the patch surface after allowing 1 minute for equilibration.

Tack Properties:

It is one of the qualitative tests applied for the determination of the tack property ofAdhesives. Simply press the thumb over the adhesive layer, and the relative tack property is determined.

Evaluation and characterization of w. coagulans transdermal patch:

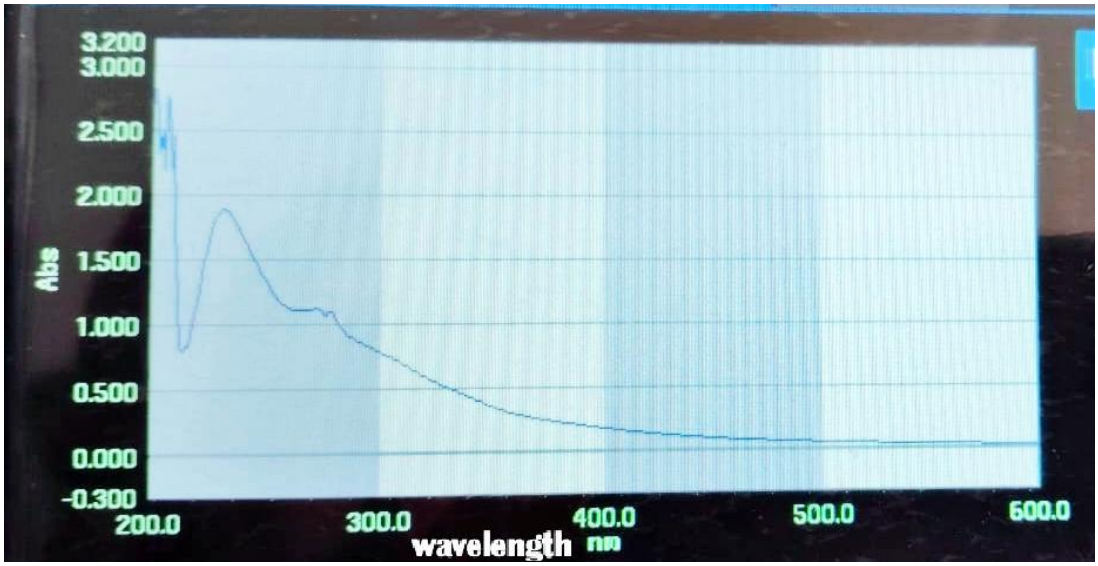
Table 3: Physical Appearance

Parameters	Observation
Color	Light Yellow
Clarity	Translucent
Texture	Smooth

Table 4: Evaluation Parameter of Transdermal Film

Properties	Observation
Thickness	0.005mm to 0.00um
Weight uniformity	0.161 to 0.16gm
Folding endurance	11 times
% of moisture content	5.59%
% of moisture uptake	3.10%
Drug content	81 (±) 1.414%
% elongation	29.51mm
Surface pH	7.9
Tack properties	Good

Selection of Wavelength:



range of

Figure 6: UV spectrum of withaniacoagulans extract

The withaniacoagulans extract stock solution of concentration 100µg/ml was scanned in the 200-600 nm for absorption maxima using double beam UV spectrophotometer. The absorption peak obtained is shown in figure:

The maximum absorption of withaniacoagulans extract was found to be at 230nm and hence it is selected as the wavelength for further studies.

Construction of calibration curve of w. Coagulans:

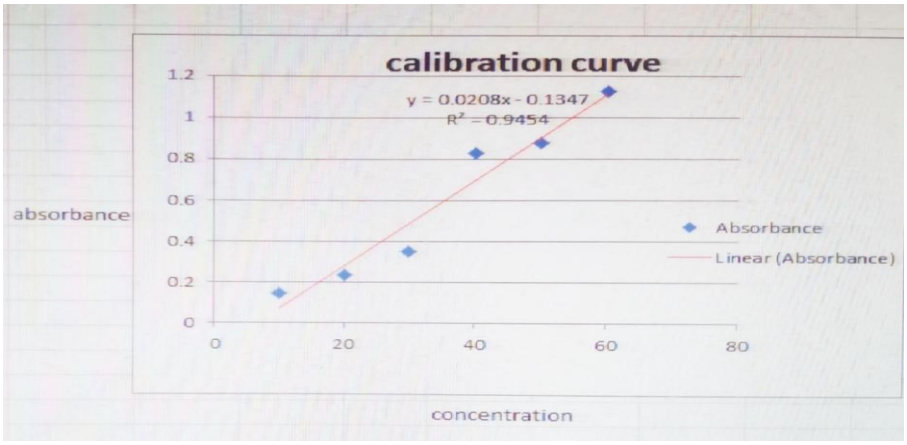


Figure 7: Calibration curve

In the calibration curve, the linearity was obtained between 10-60µg/ml concentration of withaniacoagulans and the regression value was found to be r2=0.9454.

Table 5: Calibration Curve Data

Sr. No.	Concentration (µg/ml)	Absorbance at 230nm
1.	10	0.147
2.	20	0.234
3.	30	0.354
4.	40	0.827
5.	50	0.876
6.	60	1.124

FTIR Study:

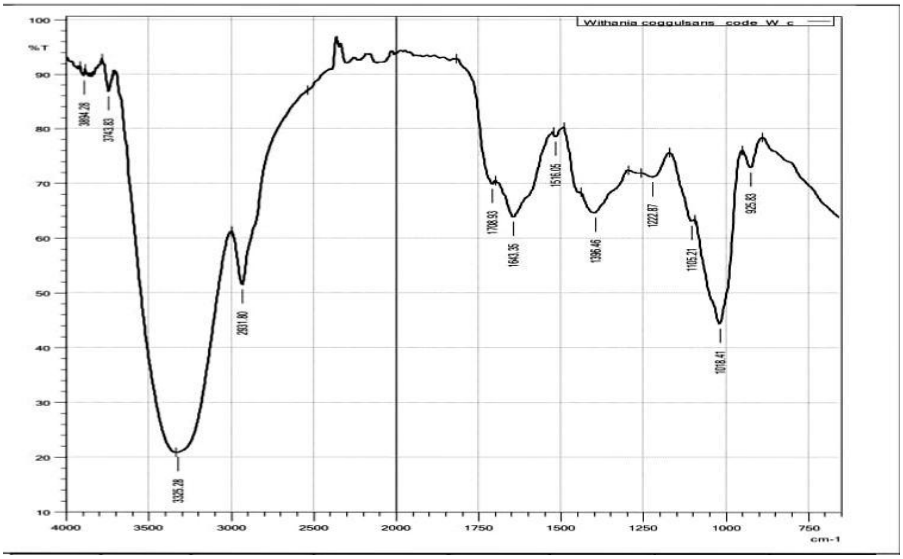


Figure 8: FTIR analysis of w. coagulansmethanolic extract

Table 6: IR Spectral data of methanolic extract of w.coagulans

Sr.no	Wavenumber cm -1	Functional group representing phytochemicals
1	925.83	Aromatic compounds represent withanolides
2	1018.41	Alcohol and phenols represents withanolides
3	1105.21	Ester group containing withanolides
4	1222.87	C-N primary amines containing withanamines
5	1396.46	Methyl group contains wthanolides
6	1516.05	C=C contains withaferin A
7	1643.35	C=O contains withanolides
8	1708.93	C=O ester containing withaferin A
9	2931.8	Alkane group containing withanolides
10	3325.28	N-H primary amines containing withanine
11	3743.83	O-H alcohol containing withanolides
12	3894.28	O-H strtchning alcohol containing withanolides

The FTIR analysis of methanolicentract of w. caogulans fruit was performed and different functional groups represented peaks at different wavenumber cm-1 were identify in table no. 11. The FTIR spectra for methanolic extract of w. coagulans were shown in figure 17. The inspection of spectra revealed that the presence of following peaks at 925.83cm-1 representing aromatic compound structure vibration wavenumber 101841cm-1 representing C-O structure vibration of alcohol, wavenumber 1105.21cm-1 representing C-O

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Ester group were characteristics of steroids. Wavemmber 1222.87cm-1 representing C-N primary amines structure, wavenumber 1396.46 represent C-H methyl group structure, at 1516.05cm-1 C-C alkene group present, wavenumber 1643.35cm-1 represents C-O structure contains carbonyl compounds, at peak 1708.93cm-1 represents carboxylic acid, at peak 2931.8cm-1 containing C-H alkyl group, wavemumber 3325.28cm-1 representing N-H primary ammes structure, at peak 3743.83cm-1 and 3894 28cm-Irepresents O-H contamungcarboxylic acid and stritching alcohol respectively.Methanolic extract of w. coagulans mainly containing withanolides and withaferine phytochemical mainly shows hypoglycemic activity

CONCLUSIONS:

In the latest research studies, it has come to see that there is a wide scope of novel drug delivery system. As we know that benefits of novel drug delivery system over the traditional drug delivery system. As it is concern with the drug delivery through the skin the transdermal drug delivery system has an effective benefit over the topical method of drug delivery.

The herbal remedies withaniacoagulans extract use in novel drug delivery to development of transdermal patches by using various polymers like HPMC. PVP. The film was found to be uniform, flexible, smooth, and transparent. The thickness of patches was found to be 0.005mm. The film was evaluated for their physical parameters-% moisture content 5.5, % moisture uptake 3.10%, uniformity content 0.161gm, folding endurance 11 times, % of elongation 29.51mm, and tack properties of formulated patch. The prepared patch shows less moisture absorption capacity and shows 5.5% absorption content. The surface PH of patch was found to be 7.9.

Study of FTIR graph of w. coagulans, extract for functional group detection. The cavaluation of withania, coagulans extract the phytochemical screening shows result the extract contains alkaloids, carbohydrates, glycosides, and flavonoids was presentFormulation of light yellow, smooth, transparent transdermal patch containing w. coagulans extract and packaging of patch with silicone backing layer.

ACKNOELEDGEMENT:

The author would like to express sincere gratitude to the management of Late Bhagirathi YashwantraoPathrikarcollege of pharmacy for their continuous support and encouragement in this research work.

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