Research Article

Formulation and Evaluation of Herbal Gel Containing the Leaf Extract of *Anacardium occidentale*

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ABSTRACT

Cashew tree has been used medicinally worldwide. Antibacterial, antifungal, antiprotozoan, antihelminthic and antiviral activities of various part extract of cashew were recorded. In this study, the antimicrobial activity of the ethanolic leaf extract of *Anacardium occidentale* was evaluated by agar well diffusion method. The extract shows best and promising activity. Now a day's topical agents are widely used to treat skin conditions. Here an attempt is made to formulate and evaluate herbal gel containing ethanolic leaf extract of *Anacardium occidentale.* Extraction is done by soxhlet hot continuous method. MIC of extract is evaluated. Optimization of gel is done by 3^2 full factorial design. Gel was subjected to evaluations such as pH, viscosity, extrudability, spreadability, drug content and skin irritation study. Stability studies were conducted as per ICH guidelines. The optimized formulation was found to be complying with all the parameters for a gel, and further the formulated gel was compared with a marketed product and the results were found to be better in all the aspect.

Keywords: Herbal gel, *Anacardium occidentale*, Antibacterial activity, Optimization.

INTRODUCTION

Today's era, use of herbal medicines become popular worldwide and are believed to be an important source of new chemical substances with potential therapeutic effects[1]. Approximately, half of the world's 25 best-selling pharmaceutical agents are derived from natural products. Cashew (*Anacardium occidentale*) tree has been used medicinally worldwide. The bark, leaves and shell oil of the plant are used medicinally to treat different ailments where the anti-bacterial, anti-fungal, anti-protozoan, antihelminthic and anti-viral activities of various part extract of cashew were documented. Different parts of the cashew plant have been used in the treatment of allergies/ inflammations, GIT syndromes, endocrine defects, cardiovascular problems, respiratory tract dysfunctions and in the treatment of cancerous growth and tumor. It has also been reported to possess anti-diabetic, anti-inflammatory and anti-ulcerogenic properties. Anti Bacterial activity of the plant is used to treat the diseases such as Leprosy, Sore throat, Syphilis, Tonsillitis, Toothache, and Venereal disease. Anti-fungal activity is used to treat the conditions such as Skin disease and dermatomycosis. Some gastrointestinal tract syndromes have also been reported to be treated using some cashew parts extracts Diarrhoea, Dysentery, Dyspepsia, Nausea, Ulcers. The cardiovascular system is not left out in the administration of cashew extracts, the antioxidant effect of flavonoids and phenolic compounds restore the heart back to normal. The respiratory tract also enjoys the effect of cashew extracts by treating cold, congestion, cough and flu. The results suggest that the extract from cashew leaves provide novel precursors for antimicrobial drug development research.[2]

It is well known that topical gels are more popular among all the topical preparations due to ease of applications. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration. For the topical treatment of dermatological diseases as well as skin care, a wide variety of vehicle ranging from solids to semisolids and liquid preparation is available to clinician and patients. Within the major group of semisolid preparations, the use of transdermal gels has expanded both in cosmetics and in pharmaceutical preparations. Transdermal application of gels at pathological sites offer great advantage in a faster release of drug directly to the site of action, independent of water solubility of drug as compared to creams and ointment[3].

PLANT PROFILE

Anacardium occidentale Linn

Figure 1: Anacardium occidentale leaf

Figure 2: Anacardium occidentale plant

Table 1: Plant Profile

Botanical name	*Anacardium occidentale* Linn.
Family	Anacardiaceae
Kingdom	Plantae
Order	sapindales
Genus	Anacardium
Subkingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Rosidae
Division	Magnoliophyta
Species	*Anacardium occidentale* L
Synonym	Cassavium pomiferum
Other names	Anacardier (French), Acajubaum, Kaschubaum (German), Cajú, cajueiro (Portugese), Anacardo (Spanish)

MATERIALS AND METHODS

Collection and Authentication of the plant material

The leaves of *Anacardium occidentale Linn* was collected from Alappuzha district, Kerala in the month of august. The sample was identified and authenticated by Dr. M.S. Francis, Associate Professor in Botany, S. H College, Thevara. After authentication, the leaves were washed with distilled water, shade dried, pulverized in a mechanical grinder and stored in a closed container till further use.

Chemicals

Ethanol; (Nice Chemicals Pvt. Ltd, Cochin), Carbopol 940; Nice Chemicals Pvt. Ltd, Cochin, Propylene Glycol; Chemdyescorporation, Gujarat, Poly Ethylene Glycol; Chemdyescorporation, Gujarat, Triethanolamine; Nice Chemicals Pvt. Ltd, Cochin, Methyl Paraben: and Chemdyescorporation, Gujarat, Propyl Paraben; Chemdyescorporation, Gujarat.

Preparation of ethanolic extract of *Anacardium occidentale Linn.* leaf extract.

The collected leaves were cleaned, and shade dried and grounded with a mechanical grinder and stored in an air tight container. It was extracted by continuous hot soxhlet extraction method. The powdered leaves (80g) were defatted with petroleum ether (60-80ºC) in soxhlet apparatus. After defatting, it was further extracted with 600ml of ethanol. The collected extracts were concentrated on rotary evaporator and stored in refrigerator until used. Obtained extracts were weighed, practical and percentage yield were calculated in terms of air dried crude material. The extract thus obtained was used to asses preliminary phytochemical screening[4,5].

Preliminary Phytochemical Screening

The presence of various phyto constituents was determined by the standard qualitative methods.

MIC of extract (Tube Dilution Method)[6]

The minimum inhibitory concentration (MIC) is the lowest concentration of a drug that prevents the growth of a particular pathogen. Determination of antimicrobial effectiveness against specific pathogen is essential for proper therapy.

In this study, tube dilution method is used to determine the MIC value in which serial dilutions of extracts were made in liquid medium which is then inoculated with standardized inoculums and incubated for a prescribed time. The lowest concentration of antibiotic/ test sample preventing the growth of organisms is considered to be the minimum inhibitory concentration.

In-vitro antibacterial activity of extract

The antibacterial activity of the *Anacardium occidentale* leaf extract was compared with the standard (Amoxicillin) against gram negative and gram positive microbial strains using agar- well diffusion method.

Pre-formulation study

a) Solubility study

Solubility is defined as the number of gram substance which will dissolve in 100 grams of solvent at a stated temperature. Solubility of the extract was observed in different solvents such as water, methanol, ethanol, 0.1N HCl, 0.1N NaoH, Distilled water, etc.

b) Determination of λmax

The absorption maximum of the extract was taken using UV double beam spectrophotometer. A solution of extract containing the concentration 10µg /ml was prepared in phosphate buffer pH 5.5 and was scanned in the range of 200-400 nm.

c) Preparation of calibration curve of extract

Accurately weighed 100Mg extract was dissolved in phosphate buffer pH 5.5 and volume was adjusted to 100ml with phosphate buffer pH 5.5 to produce stock solution of 1000µg/ml. from the stock solution 1oml was taken in to a 100 ml volumetric flask and made up to the volume with phosphate buffer pH 5.5 to get a concentration of 100µg/ml. From this stock solution aliquots of 0.2, 0.4, 0.6, 0.8 ml were transferred into a separate series of 10 ml volumetric flask and made up to volume with phosphate buffer pH 5.5, to get

concentrations of 2,4,6,8,10 µg /ml solutions respectively. The absorbance was measured at 241.5 nm against blank. From the absorbance values calibration curve of extract was plotted.

Preparation of Gel

Procedure

Accurately weighed quantity of Carbopol 940 was dispersed in 50 ml of distilled water with continuous stirring. The 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. The solution was cooled, and then propylene glycol 400 and polyethylene glycol 200 were added. Further required quantity of ethanolic extract of *Anacardium occidentale* were mixed to the above mixture and volume was made up to 100 ml by adding remaining distilled water. Finally, all ingredients were mixed properly to the Carbopol 940 gel with continuous stirring and Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency[7].

Table 2: Formulation chart

No.	Contents	Quantity (100 gm)
1	Carbopol 940	Q.S
2	Methyl paraben	0.15 gm
3	Propyl paraben	0.03 gm
4	Propylene glycol	5.0 ml
5	Polyethylene glycol	15.0 ml
6	Extract	Q.S
7	Distilled water	Upto100ml

Optimization by 3^2 full factorial design

A 3^2 Full factorial design was constructed where the amounts of carbopol 940 (X_1) and concentration of drug extract (X_2) where selected as the two independent variables. The levels of the two factors are selected on the basis of preliminary studies carried out before implementing the experimental design. All other formulation and processing variable were kept constant throughout the study. Optimization of preparation was done by design expert software version10 (version 10, stat-ease inc.., and Minneapolis, MN). All the formulations were prepared and evaluated for various parameters and the effects of gelling agent and drug concentration was studied. The data was inputted to design expert software and polynomial equation was obtained. The responses (dependent variables) studied were antimicrobial activity and viscosity of the formulation[8].

Table 3: 3^2 full factorial design layout

Factors Independent variables	Coded levels			Responses Depended variables
	-1	0	1	
X_1(carbopol Conc %)	1.5	2	2.5	Y1- viscosity of gel
X_2(drug Conc %)	2.5	5	10	Y2- antibacterial activity of gel

Evaluation of Optimized Gel[9,10].

a) Physical appearance

The physical appearance was visually checked for the colour, appearance, and the feel of application.

b) pH determination

The pH of the gel was determined by using the digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. Electrodes were completely dipped in to the gel formulation and pH was noted. The measurement of pH of formulation was done in triplicate and average values were calculated.

c) Extrudability determination.

The gel formulations were filled in to a collapsible metal tube. The tubes were pressed to extrude the material and the extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5cm ribbon of gel in 10 seconds.

d) Viscosity determination

The viscosity of the prepared gel formulation was measured by Brookfield viscometer by selecting the spindle number and rpm. 40gm of preparation was kept in 50ml beaker which was set till spindle groove was dipped and rpm was set and dual reading was measured after three minutes. From the reading obtained, the viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as means.

e) Spreadability

It indicates the extent of area to which a gel readily spreads on application to skin or affected part. The therapeutic potency of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel which is placed in between the slides under the direction of certain load. Lesser the time taken for the separation of two slides, better the Spreadability. It is calculated by using the formula.

S = M.L / T. where

M = weight tied to upper slide.

L = Length of glass slide

T = Time taken to separate the slides.

RESULTS AND DISCUSSION

The preliminary phytochemical studies of extract revealed the presence of carbohydrates, tannins, saponins, glycosides, resins, flavonoids and alkaloids. Solubility study shows that it is very soluble in ethanol and Dimethyl sulphoxide. Standard solution of extract (10µg/ml) was scanned between 200-400 nm in UV spectrometer using phosphate buffer as solvent. Wavelength at which maximum absorption occurs was found to be 271.5 nm. The standard calibration curve for extract was prepared in

phosphate buffer pH 5.5 at the λ max271.5 nm. The calibration curve obtained is linear.

Formulation and Optimization of Antibacterial AO Gel

9 formulations were prepared by using carbopol, poly ethylene glycol, propylene glycol and extract. Concentration of carbopol and concentration of extract were varied in 9 formulations.

Development of the optimum batch

Based on the statistical evaluations the software provided 100 solutions from which software selected one optimum batch. The formula for the optimum batch is given in table 8.

Table 4: MIC value of extract

Organism	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml
Bacillus subtilis	Extract	-	-	-	-	+	+	+
	Std	-	-	-	-	-	-	+
S.aureus	Extract	-	-	+	+	+	+	+
	Std	-	-	-	-	-	-	+
E.coli	Extract	-	-	-	-	+	+	+
	Std	-	-	-	-	-	-	+
Pseudomonas aeuroginosa	Extract	-	-	-	+	+	+	+
	Std	-	-	-	-	-	+	+

Table 5: Antibacterial activity of extract

Organism	Zone of inhibition (mm)			
	Standard		Ethanolic Extract	
	50µg	100 µg	150 µg	200 µg
Bacillus subtilis	32	22	25	28
S.aureus	36	15	26	26
E.coli	21	11	16	18
Pseudomonas aeuroginosa	34	16	27	28

Table 6: Formulation chart

Formulation code	Carbopol (g)	Extract (%)	Methyl paraben(g)	Propyl paraben	Polyethylene glycol (ml)	Propylene glycol (ml)	Triethanolamine	Distilled water
F1	1.5	2.5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F2	1.5	5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F3	1.5	10	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F4	2	2.5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F5	2	5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F6	2	10	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F7	2.5	2.5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F8	2.5	5	0.15	0.03	15.0	5.0	Q.S	Upto100ml
F9	2.5	10	0.15	0.03	15.0	5.0	Q.S	Upto100ml

Table 7: Observed responses in 9 experimental run

Formulation code	Independent variables		Response variables	
	X1(g) Concentration of carbopol	X2(%) Concentration of extract	Y1(cp) viscosity	Y_2 (mm) Antibacterial activity of gel
F1	1.5	2.5	21154	32
F2	1.5	5	21243	33
F3	1.5	10	21234	31
F4	2	2.5	24546	28
F5	2	5	24576	27
F6	2	10	24543	25
F7	2.5	2.5	26564	23
F8	2.5	5	26457	24
F9	2.5	10	26376	20

Table 8: Formula for Optimum batch

Number	Carbopol*	Extract*	Viscosity	Antibacterial activity	Desirability
1	*1.722*	*5.537*	22882.851	29.790	1.000

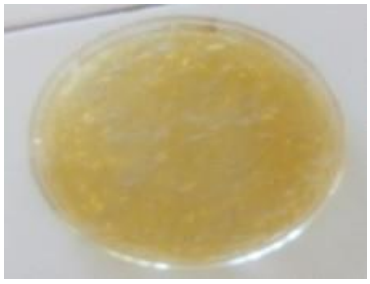

Figure 3: Optimized A.O gel

The evaluation studies show that it is a yellow colored non transparent thick gel. pH found to be 7. Viscosity was 2280 cps. Spreadability and extrudability were found to be good. The result of skin irritation study gives a score of zero. It is as certain that there is no skin irritation in A.O gel.

Antibacterial activity of Gel

The antibacterial activity of AO gel was compared with marketed formulation of Ofloxacin (EXOCIN) against gram positive and gram negative organisms. The results were found to be better in all the aspect.

CONCLUSION

Herbal medication is considered safer than allopathic medicines due to its lesser side effects and low cost. The gel formulation can provide better absorption characteristics and hence the bioavailability of drug. It also provides the better information regarding to the formulation and evaluation parameters and to provide the better therapeutic effects to patient compliance.

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