IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES OF PLANT EXTRACT (ROOT) OF LEUCAS ASPERA

[1]G. Vijaya sindhuri, [2]Ravi kumar, [3] Naveen Kumar, [4]Likhitha Reddy, [5] Vaishnavi

Corresponding Author: Gopu Vijaya sindhuri *

[1]Associate Professor,
Department of Pharmaceutics
St. Mary's College of Pharmacy, Secunderabad, Telangana, India

Abstract: The purpose of this investigation is to investigate the physicochemical evaluation and anti-microbial activity of plant extract of leucas aspera root by using methanol and ethyl acetate in 1:1 ratio. The primary phytochemical analysis of root extract of leucas aspera showed the presence of alkaloids, glycosides, tannins, flavonoids, terpenoids, saponins. Six batches of H1, H2, H3, S4, S5& S6 Plant extract (root) of Leucas aspera, Transdermal patches were prepared by solvent casting technique. From the results of invitro diffusion and physicochemical studies, H2 & S5 was concluded as best formulation. Then they were subjected to screening of anti- microbial activity. The anti-microbial screening result showed that the H2 was highly inhibiting the microbial growth around the patch and additional research on these plants could lead to the discovery of novel bioactive chemicals.

Index Terms **-** Transdermal drug delivery, Leucas aspera, Plant extract, Anti-microbial action**.**

I. INTRODUCTION

Traditional system of medicine is one of the centuries old practices and long-serving companion to humankind in the fight against disease and in leading a healthy life. Indigenous people have been using the unique approach of their traditional system of medicine for centuries and among the most renowned are the Chinese, Indian, African systems of medicine. Traditional medicine refers to any ancient and culturally based healthcare practice differing from scientific medicine and is largely transmitted orally by communities of different cultures.[1] The World Health Organization (WHO) observes that it is difficult to assign one definition to the broad range of characteristics and elements of traditional medicine, but that a working definition is essential. It thus concludes that the traditional medicines ''[include] diverse health practices, approaches, knowledge, and beliefs incorporating plant, animal and mineral based medicines, spiritual the rapies, manual techniques, and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.[2]

Several developed countries have a major proportion of the population that uses traditional practice of health, especially medicinal plants, and have taken steps to preserve its popularity for historical and cultural reasons. Moreover, it has been reported that more than70%of the developing world's population still depends on the complementary/alternative systems of medicine, otherwise known as traditional medicine, for example, up to 80%of the population in Africa,71%in Chile, and 40% in Colombia, and others.[3]

The modern health care service has posed immense threat to indigenous health practices because of their potential and speedy therapeutic effect. Also, traditional systems are undervalued by the people. However, the rise in population, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments.[10]

II .MATERIALS AND METHODS:

List of Chemicals

Table No.1. List of Chemicals

S.No.	Chemical Name	Company Name
1.	HPMC	Fine chem industry, Mumbai
2.	Sodium alginate	Finer limited,Ahmedabad
3.	Oleic acid	P &G Ltd. (Merck), Mumbai
4.	Glycerin	P & G Ltd. (Merck), Mumbai
5.	Potassium dihydrogen orthophosphate	MicrofineChemicals, NewDelhi
6.	Ethyl acetate $ petroleum ether	MicrofineChemicals,NewDelhi

2.1. Pre formulation Studies:

Preformulation testing is the first step in the rational development of dosage forms of drugs substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be man produced. The following Preformulation studies are carried out

- Physical appearance
- Solubility
- Standard curve

2.2.Collection of Leucas aspera plant root:

Fresh plant root of Leucas aspera was collected from the natural habitat of Government Arts college campus, kamareddy during the month of April 2022.

The roots of the leucas aspera were washed thoroughly three times with water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were sealed in separate polythene bags, until the time of extraction.

Fig No:1 Crude drug extract

2.2.1.PREPARATION OF CRUDE EXTRACT

The dried root materials were pulverized into fine powder using a grinder.

About forty grams of powered roots were extracted successively with 200ml of aqueous (97^0-103^0 C), ethyl acetate (77^0-85^0 C) and petroleum ether (62^0-66^0 C) in soxhlet extractor until the extract was clear. The solvent extracts were evaporated by using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4^o C for further use.

Extraction refers to process for the isolation of the active ingredients from the drug material.

Two methods are used for extraction

1.Maceration

2.soxhlet apparatus

1.Maceration

- 20g of powdered drug is taken in a beaker
- 250 ml of methanol is added
- And it is kept on magnetic stirrer for proper mixing and soaked for 4 days
- After complete soaking it is filtered, and the resultant residue is stored for further process.

2.Soxhlet

- Soxhlet apparatus is setup.
- Thimble is prepared and 5g of the powdered drug is taken.
- Solvents are taken in the ratio Ethyl Acetate: Petroleum Ether,1:1 ratio.
- It is kept for 6 hrs. for extraction process.
- It is then filtered, and the extract is used for phytochemical screening, formulation studies and evaluation studies

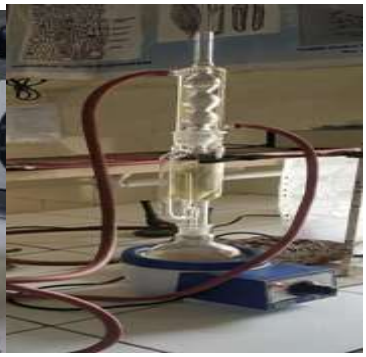


Fig:2 preparation of crude drug extract **Fig:3 soxhlet extraction process**

2.3 Phytochemical Studies [38,39]

The plant extract was subjected to Phyto chemical studies to find out the presence and absence of constituents.

Table No.2: Phytochemical Test

EXPERIMENT	OBSERVATION	INFERENCE
Test for Alkaloids		
1.Dragendroff'stest: The extract was treated with Dragendroff' s reagent (potassium bismuth iodide solution)	Orange, b r o w n Precipitate was formed	Presence of alkaloids
2.Mayers'reagent's: The extract was treated with Mayer's (potassium mercuric iodide solution) Reagent	Precipitate formed	Presence of alkaloids
3.Wagner'sreagent: The extract was Treated With wagner's reagent (iodide and potassium Tri iodide solution)	Reddish brown Precipitate Was formed	Presence of alkaloids
Test for Glycosides		
1.Brontragers test: To the extract add dilute H2SO4 and filtered. Filtrate Was extract withLittle chloroform layer was Separated out and add equal Volume of dilute NH3.	Red color observed in Ammoniacal layer	Presence of glycosides
Test for Saponin glycosides		
1.Foamtest: Shake the extract With water.	Foam was produced/formed	Presence of saponin Glycosides
Test for Tannins and Phenolic compounds		
1.Ferricchloridetest: To the aqueous extract few drops of ferric chloride solution were added	Dark black color formed	Presence of tannins and phenolic compounds

2.Brominewatertest: To the aqueous extract is treated with bromine water	Discoloration of bromine Water	Presence of tannins and phenolic compounds
3.KMnO4test: To the aqueous extract is treated with dilute KMnO4.	Discoloration of solution	Presence of tannins and phenolic compounds.

2.4. Preparation of Transdermal Patch [40]

Six batches of plant extract of Leucas aspera root of transdermal patches were prepared using drug with two different polymers in three different ratios (1:4,1:6 &1:8). Weighed quantity of polymer was dissolved in calculated quantity of water and heated on a water bath. Calculated amount of extract was added to the above mixture and stirred well until a homogenous mixture was formed. Then calculated amount of permeation enhancer and glycerin were added. In all the six batches the quantity of extract was same.

The resultant mixture was poured into a Petri dish and air dried at room temperature for 24h.The patches were then peeled off from the Petri dish with the help of a knife and kept in desiccator.

Table No.3: Formula for TDDS

Ingredients	**Formulation Code**					
	TH1	**TH2**	**TH3**	**TS4**	**TS5**	**TS6**
Extract(mg)	40	40	40	40	40	40
HPMC (mg)	160	240	320	-	-	
Sodium alginate(mg)	-	-	-	160	240	320
Oleic acid(ml)	0.3	0.3	0.3	0.3	0.3	0.3
Glycerin(ml)	0.3	0.3	0.3	0.3	0.3	0.3
Water	q.s	q.s	q.s	q.s	q.s	q.s

2.5. Preparation of Calibration curve of *Leucas aspera plant* Extract

Accurately weighed quantity(100mg) of extract was transferred in to a100ml volumetric flask and dissolved in small amount of distilled water (D.W) and made up to the volume to make thestandardstocksolutionof1mg/ml.

From the stock, 1ml was taken in 10ml volumetric flask and made up the volume with the buffer; from this solution 0.5ml to 3ml solution was transferred to10ml volumetric flask and made up to required volume with more D.W and the resulting concentration ranges from5to50µg/ml. The absorbance of these solutions was determined at 382nm using UV spectrophotometer. The calibration curve was constructed between the absorbance and concentration.

2.6.Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared as per the method described in I.P 1996using disodium hydrogen phosphate and sodium hydroxide. The pH was adjusted to 7.4priortoquantitativeestimation.

2.7. Physicochemical evaluation of *Leucas aspera* Transdermal patch [41,42]

Formulated patches were subjected to the preliminary evaluation tests. Patches with any imperfections, entrapped air, or differing in thickness, weight(or)content uniformity were excluded from further studies.

2.8.Thickness of the Patch

The thickness of the patch was assessed by using digital vernier caliper at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined.

2.9.Folding Endurance

This was determined by repeatedly folding one patch at the same place till it broke. The number of times the patch could be folded at the same place without breaking gave the value of folding endurance.

3.Percentage Moisture uptake

Thepatchwereweighedaccuratelyandplacedindesiccatorscontainingaluminium chloride. After 24 h, the patch was taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight. With respect to initial weight. It is calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

3.1.Percentage Moisture content

The patch was weighed and kept in desiccators containing calcium chloride. After 24h the patch was taken out and weighed. The percentage moisture content was calculated using the following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

3.2. Determination of surface pH

The patches were allowed to swell by keeping them in contact with 1ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1min.

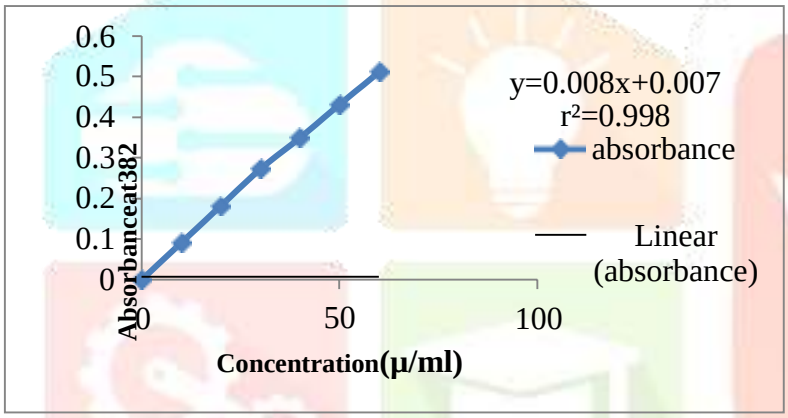
4. Screening of Antimicrobial activity of *Leucas aspera*.

Anti-Bacterial Activity Principle

Discs impregnated with known concentration of antibiotics discs are placed on agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18-24 hrs. at 37oC. During this period, the antibacterial agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Concentration(µg/ml)	Absorbance 382nm
	Average±SD
0	0.000±0.000
10	0.091±0.001
20	0.182±0.001
30	0.273±0.001
40	0.363±0.001
50	0.432±0.001
60	0.512±0.001

Table No.4: Standard values of Extract of Leucas aspera



FigNo.4: Standard Curve of Leucas aspera

III .RESULTS AND DISCUSSIONS

3.1 Standard Curve values of Extract of *Leucas aspera*

3.2. Physical appearance

Color : Green
Taste : Bitter taste
Solubility : Freely soluble in Distilled Water

3.3 Phytochemical Studies

TableNo.5: Phyto chemical constituents

S.No.	Chemical constituents	Aqueous Extract
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Phenolic	+
5.	Compounds	+
6.	Flavonoids	+

Mean ± S.D: n=3

(+) Presence of constituent (-) Absence of constituents

The Phyto chemical studies revealed that the presence of alkaloids, saponins, tannins, phenolic compounds, flavonoids, and sterol.

The six (H1, H2, H3, S4S5, S6) batches of extract loaded patches with different ratios of two different polymers were subjected to various physico chemical evaluations.

Based on thickness, uniformity of weight, folding endurance, percentage moisture uptake moisture content the formulations H2 and S5were selected for further studies.

Formulation code	Uniformity of weight(g)	Thickness(mm)	Drug content(%)	FoldingEndurance(no's)	Moisture Uptake(%)	Moisture Content(%)	SurfacepH
Transdermal Patch of HPMC(H)	H1	0.38±0.14	0.43±0.73	85.23±0.92	235±0.76	2.14±0.08	2.866±0.07
	H2	**0.46±0.85**	**0.49±0.23**	**85.69±0.56**	**249±0.23**	**2.85±1.03**	**3.422±0.22**
	H3	0.49±0.10	0.50±1.13	88.98±0.16	254±0.36	3.79±1.03	3.940±0.36
Transdermal Patch of Sodium alginate (S)	S4	0.39±0.43	0.36±0.33	82.03±0.22	241±0.72	2.75±0.65	1.736±0.46
	S5	**0.41±0.65**	**0.45±0.76**	**83.35±0.94**	**265±0.44**	**1.97±0.44**	**1.657±0.03**
	S6	0.48±0.50	0.47±0.16	80.13±0.40	247±0.42	2.79±0.35	2.457±0.03

3.4.Optimized formula of *Leucas aspera (root) of* Transdermal patch

TableNo.6: Optimized formula of *Leucas aspera (root) of* Transdermal patch

S.NO.	Ingredients	H (HPMC)	S (Sodium alginate)
1.	Extract(mg)*Leucas aspera*	40	40
2.	Polymer(mg)	240	240
3.	DMSO/SLS (ml)	0.3	0.3
4.	Glycerin(ml)	0.3	0.3
5.	Water(ml)	q.s	q.s

3.5 Physicochemical Evaluation of Selected formulations

Table No. 7: Uniformity of weight

S. No.	Formulation code	Weight(g)
1.	**HPMC(H2)**	**0.46±0.85**
2.	Sodium alginate(S5)	0.41±0.65

Mean ± S.D : n=3

TableNo.8: Thickness of the patch

S. No.	Formulation code	Thickness(mm)
1.	**HPMC (H2)**	**0.49±0.23**
2.	Sodium alginate(S5)	0.45±0.76

Mean±S.D:n=3

Table No. 9: Determination of Drug content

S. No.	Formulation code	% Drug content
1.	**HPMC(H2)**	**85.69±0.56**
2.	Sodium alginate(S5)	83.35±0.94

Mean±S.D:n=3

TableNo.10: Folding Endurance.

S.No.	Formulationcode	Folding Endurance
1.	**HPMC (H2)**	**249±0.23**
2.	Sodiumalginate(S5)	245±0.44

Mean±S.D:n=3

Table No. 11: Percentage Moisture Uptake.

S. No.	Formulation code	%Moisture Uptake

1.	**HPMC (H2)**	**2.85±1.03**
2.	Sodiumalginate(S5)	1.97±0.44

Table No. 12: Percentage Moisture Content

S.No.	Formulation code	%MoistureContent
1.	**HPMC (H2)**	**3.422±0.22**
2.	Sodium alginate(S5)	1.657±0.03

Table No.13: Surface pH

S. No.	Formulation code	Surface pH of patches
1.	**HPMC(H2)**	**7.3±0.72**
2.	Sodiumalginate(S5)	7.1±0.12

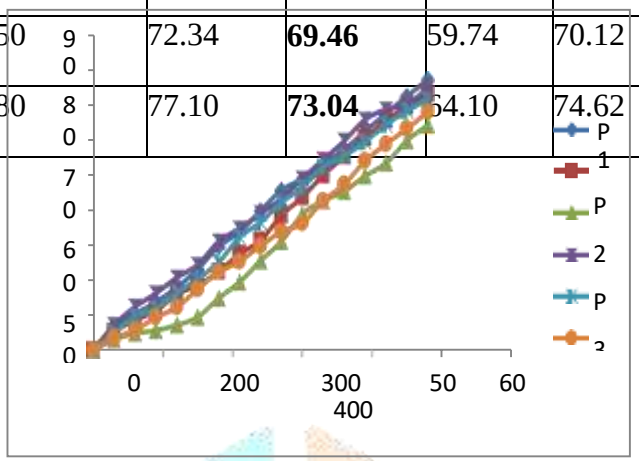
Mean± S.D: n=3

***4. Invitro* drug diffusion study**

TableNo.14: *Invitro* drug diffusion study

Time in(min)	%Drug diffusion					
	HPMC (H)			Sodium Alginate(S)		
	H1	H2	H3	S4	S5	S6
0	0	**0**	0	0	**0**	0
30	6.78	**5.2**	2.98	7.15	**4.86**	3.46
60	10.30	**8.6**	4.78	12.36	**9.5**	5.72
90	13.65	**11.21**	5.64	16.19	**11.98**	9.34
120	17.89	**15.96**	7.15	20.72	**16.28**	12.20
150	23.10	**18.54**	9.45	24.37	**20.41**	17.40
180	29.54	**22.34**	14.82	30.97	**25.01**	22.19
210	33.68	**27.45**	19.38	34.65	**32.21**	25.30
240	39.70	**31.23**	25.48	38.98	**36.43**	29.68
270	45.58	**38.42**	30.98	43.12	**42.11**	33.76
300	48.77	**43.58**	38.73	48.94	**46.36**	36.15
330	53.22	**49.86**	42.54	54.30	**52.21**	42.56

360	57.10	**55.25**	45.24	59.61	**55.34**	47.26
390	62.41	**60.46**	49.64	65.73	**59.43**	53.90
420	67.38	**65.46**	53.28	68.60	**64.22**	58.76
450	72.34	**69.46**	59.74	70.12	**68.18**	63.12
480	77.10	**73.04**	64.10	74.62	**71.46**	67.92

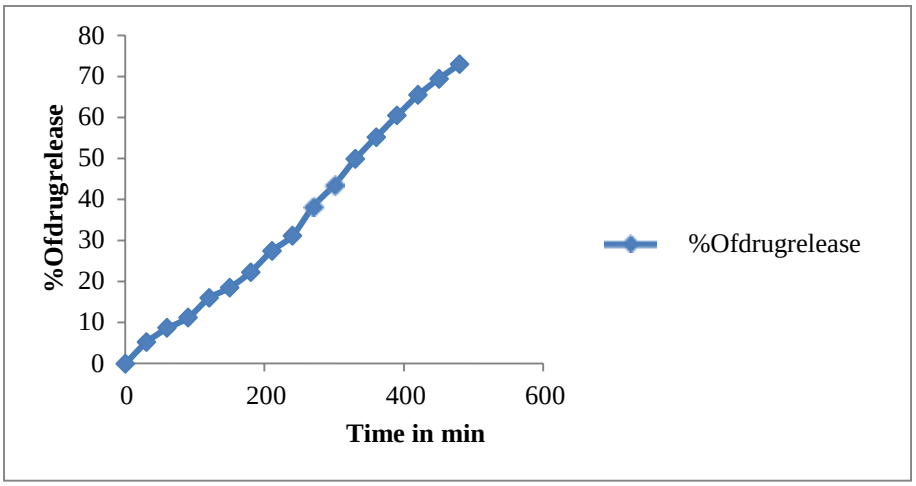


FigNo.5: In vitro drug diffusion study

TableNo.15: Invitro drug diffusion profile of H2

S. No.	Time(min)	%Drug diffusion ofH2
1.	0	0
2.	30	5.2±0.23
3.	60	8.6±0.45
4.	90	11.21±0.44
5.	120	15.96±0.76
6.	150	18.54±1.78
7.	180	22.34±0.59
8.	210	27.45±1.23
9.	240	31.23±0.61
10.	270	38.42±1.24
11.	300	43.58±1.90
12.	330	49.86±1.54
13.	360	55.25±0.23
14.	390	60.46±1.34
15.	420	65.46±0.91
16.	450	69.46±0.72
17.	480	73.04±0.61

Mean±S.D: n=3

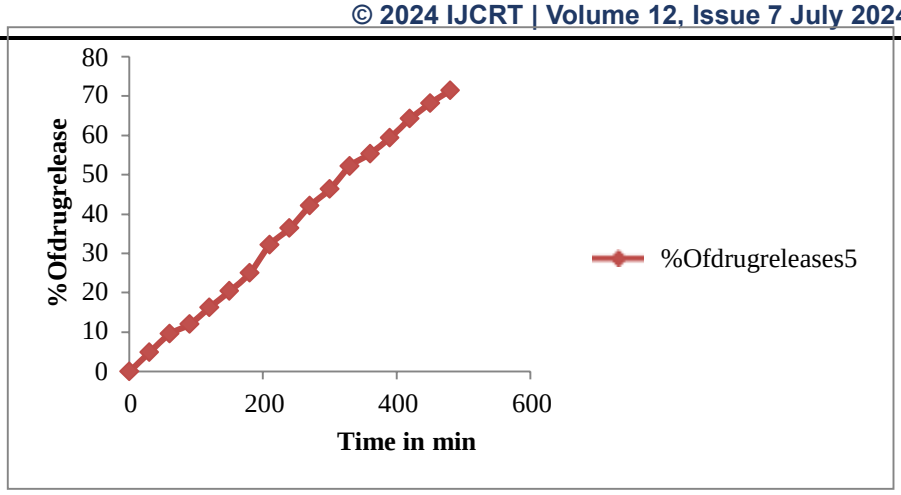


FigNo.6: In vitro drug diffusion profile of H2

TableNo. 16 : *Invitro* drug diffusion profile of S5

S. No	Time(min)	%Drug diffusion of S5
1	0	0
2	30	4.86±0.23
3	60	9.95±0.45
4	90	11.98±0.44
5	120	16.28±0.76
6	150	20.41±1.78
7	180	25.01±0.59
8	210	32.21±1.23
9	240	31.23±0.61
10	270	42.11±1.24
11	300	46.36± 1.90
12	330	52.21±1.54
13	360	55.34±0.23
14	390	59.43±1.34
15	420	64.22±0.91
16	450	68.18±0.72
17	480	71.46±0.61

Mean S.D: n=3

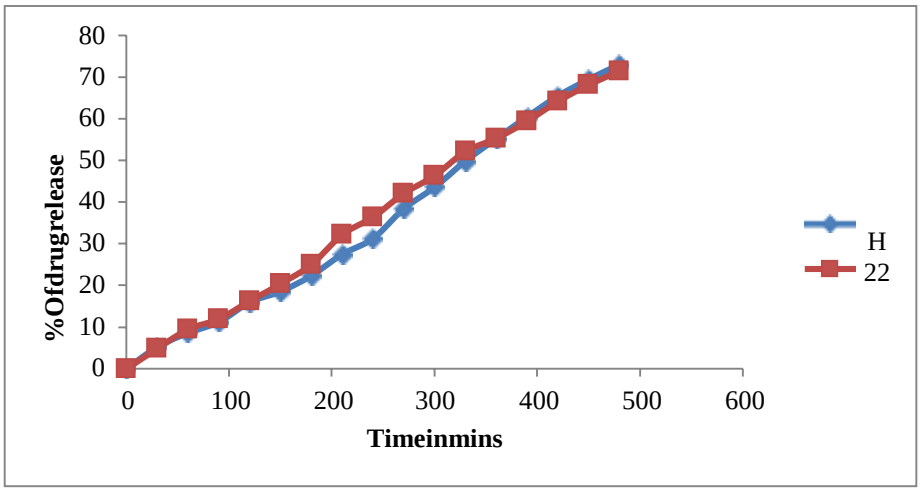


FigNo.7: *Invitro* drug diffusion profileofS5

TableNo.17: Comparative invitro drug diffusion profile

S. No.	Time(min)	%Of drug diffusion H2	%Of drug diffusion S5
1	0	0	0
2	30	5.2±0.23	4.86±0.23
3	60	8.6±0.45	9.95±0.45
4	90	11.21±0.44	11.98±0.44
5	120	15.96±0.76	16.28±0.76
6	150	18.54±1.78	20.41±1.78
7	180	22.34±0.59	25.01±0.59
8	210	27.45±1.23	32.21±1.23
9	240	31.23±0.61	31.23±0.61
10	270	38.42±1.24	42.11±1.24
11	300	43.58±1.90	46.36±1.90
12	330	49.86±1.54	52.21±1.54
13	360	55.25±0.23	55.34±0.23
14	390	60.46±1.34	59.43±1.34
15	420	65.46±0.91	64.22±0.91
16	450	69.46±0.72	68.18±0.72
17	480	73.04±0.61	71.46±0.61

MeanS.D:n=3



FigNo.8: Comparative invitro drug diffusion profile

The selected 2 batches of formulations HPMC (H2), Sodium alginate (S5) was subjected comparative *in vitro* permeation studies. Formulation P2 showed sustained diffusion in a controlled manner up to 8hrs.

5.Screening of Anti-microbial activity of Plant extract of Leucas aspera

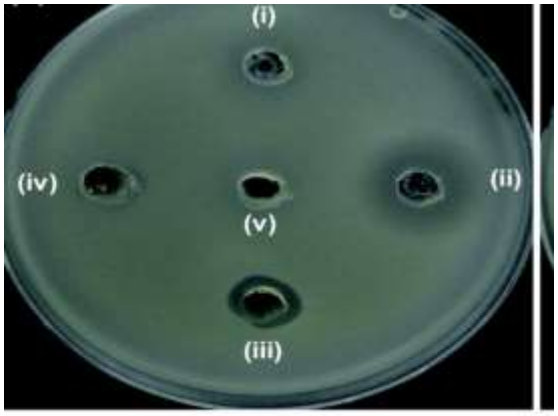
The anti - microbial activity for the given sample was carried out by cup plate method (IndianPharmacopoeia1996, volIIA-105). The test microorganism of *Staphylococcus aureus*, *E.Coli*wereobtainedfromSt Francis college secunderabad and maintained by periodical sub culturing on nutrient agar medium for bacteria. The effect produced by the sample was compared with the effect produced by the positive control.

5.1. For Bacteria

After 24h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the patch.

GRAM POSITIVE BACTERIA

Fig:9. Staphylo coccus aureus



GRAM NEGATIVE BACTERIA

Fig10 :E. Coli

Table No: 18 . Screening of Antimicrobial activity

S.No.	Name of the microorganism	A	B	C	D
1.	*Staphylococcusaureus*	9	-	8	-
2.	*E.Coli*	7	-	6	-

A: HPMC (H2); B: HPMC without drug patch ; C: Sodium alginate (S5); D: sodium alginate without drug When compared to S5, H2 showed greater inhibition against Staphylococcus aureus.

IV. SUMMARY AND CONCLUSION

Six batches of H1, H2, H3, S4, S5&S6) Plant extract (root) of Leucas aspera, Transdermal patches were prepared by solvent casting technique. The various formulation parameters, Drug-Polymer ratios and permeation enhancers ere optimized to get thin, transparent, smooth, stable, and high permeable transdermal patches. From the optimization, best 2 formulations H2 & S5 were selected based on physicochemical evaluation and *invitro* drug diffusion study 0.3ml of glycerin was added as plasticizer to produce a flexible patch without having major influence on their diffusion property. If the amount exceeds, the film loses its flexibility and become stiff. The plasticizer diffuses through the patch and softens the polymer particles. This softening promotes latex coalescence and patch formation. All the six batches were evaluated for Percentage Moisture uptake, Percentage Moisture content, Thickness, Folding Endurance, Percentage Drug content. The formulations H2 & S5 showed maximum % Moisture uptake, Moisture content, Thickness, folding endurance, % Drug content. No significant difference in drug content was observed between the patches among the six formulations. This indicates the homogenous dispensing of drug during the patch preparation. From the results of *invitro* diffusion and physicochemical studies, H2 & S5 was concluded as best formulation. Then they were subjected to screening of anti-microbial activity The anti-microbial screening result showed that the H2 was highly inhibiting the microbial growth around the patch. The present work has achieved the objectives of formulation of transdermal patch of Plant extract of Leucas aspera by using different polymers.

V. ACKNOWLEDGMENT

I would like to express my sincere appreciation to the St. Marys college of Pharmacy , Secunderabad and individuals whose contributions and support have greatly enhanced the quality and rigour of this research. First and foremost, I am grateful to my primary advisor Vijaya sindhuri for her unwavering guidance, insights, and constant encouragement throughout the research period. her expertise and wisdom were an invaluable asset to this project. The authors are thankful to Director Fr. Y.Bala Reddy for offering facilities and resources for this project. His support facilitated the smooth execution of the research. Lastly, I am thankful to my family for their understanding, encouragement, and support.

VI. REFERENCES

1. Prashant KR, Dolly J, Singh KR, Gupta KR, Watal G. Glycemic properties of *Trichosanthes dioica* leaves. *Pharm Biol.* 2008;46(12):894–899.
2. Jigna P, Sumitra C. *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae) *Afr J Biomed Res.* 2006;9:89–93.
3. Morales G, Paredes A, Sierra P, Loyola LA. Antioxidant activity of 50% aqueous-ethanol extract from *Acantholippiadeserticola*. *Biol Res.* 2008;41(2):151–155.
4. Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J Ethnopharmacol.* 2003;85:231–235.
5. Gani A. *Chemical constituents and uses.* Dhaka: Asiatic Soc of Bangladesh; 2003. Medicinal plant of Bangladesh; p. 277.
6. Goudgaon NM, Basavaraj NR, Vijayalaxmi A. Antiinflammatory activity of different fractions of *Leucas aspera* Spreng. *Indian J Pharmacol.* 2003;35:397–398.
7. Srinivas K, Rao MEB, Rao SS. Anti-inflammatory activity of *Heliotropium indicum* Linn and *Leucas aspera* Spreng in albino rats. *Indian J Pharmacol.* 2000;32:37–38.
8. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. *Chem Pharm Bull.* 2003;5:595–598.
9. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Diterpenes from *Leucas aspera* inhibiting prostaglandin-induced contractions. *J Nat Prod.* 2006;69:988–994.
10. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian medicinal plant. National institute of science communication and information resources.* New Delhi: Council of Scientific and Industrial Research (CSIR); 2002. p. 153.
11. Mangathayaru K, Thirumuragan D, Patel PS, Pratap DVV, David DJ, Karthikeyan J. Isolationand identification of nicotine from *Leucas aspera* (wild) link. *Indian J Pharm Sci.* 2006;68:88–90.
12. Rahman MS, Sadhu SK, Hasan CM. Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucasaspera* root. *Fitoterapia.* 2007;78:552–555.
13. Oktay M, Gulcin I, Kulfrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. *LebensonWiss Technol.* 2003;36:263–271.
14. Sein TT, Spurio R, Cecchini C, Cresci A. Screening for microbial strains degrading glass fiber acrylic composite filters. *Int Biodeterior Biodegradation.* 2008;63:901–905.
15. NCCLS . *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.* 3rd ed. Wayne, PA: NCCLS; 2002. pp. M100–S12.
16. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 1982;45(5):31–34.
17. Rosy BA, Joseph H, Rosalie Phytochemical, pharmacognostical, antimicrobial activity of *Indigofera spalathoids* Vahl., (Fabaceae) *Int J Biol Technol.* 2010;1:12–15.
18. Zuraini Z, Yoga Latha L, Suryani S, Sasidharan S. Anticandida albicans activity of crude extract of the local plant, winged beans leaf. *Plant Kuala Lumpur.* 2007;80:653–657.
19. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, et al. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia*

caryophyllata (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem) *J MicrobiolAntimicrob.* 2011;3(1):1–7.

20. Srinivasan D, Sangeetha N, Suresh T, Perumalsamy PL. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J Ethnopharmacol.* 2001;74:217–220.

21. Mangathayaru K, Lakshmikant J, Shyam SN, Swapna R, Grace XF, Vasantha J. Antimicrobial activity of *Leucas aspera* flowers. *Fitoterapia.* 2005;76:752–754.

22. Geran RI, Greenberg HM, McDonald M, Abbott BJ. Protocol for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rep.* 1972;33:1–17.