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FORMULATION AND EVALUATION OF AN ANTIFUNGAL POLYHERBAL GEL: AN ALTERNATIVE TREATMENT FOR VAGINAL INFECTION

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Abstract
Thuja orientalis, Punica granatum and *Aloe barbadensis miller* are well known traditional medicinal herbs for antifungal activity. The combination of these herbs has not been studied so far for its activity against vaginitis. Hence, the present study was designed to evaluate anti-fungal activity of polyherbal gel formulations for vaginal infection with a combination of ingredients such as *Thuja orientalis, Punica granatum, Aloe barbadensis miller* and Coconut oil. In this present study, polyherbal formulation was prepared using methanolic extracts of *Thuja orientalis* and *Punica granatum,* fresh leaves of *Aloe barbadensis miller* and Coconut oil by incorporating with Carbopol 934 as a gelling agent. The prepared polyherbal formulations were evaluated with various parameters which includes organoleptic, pH, extrudability, Spreadbility, homogeneity, grittiness and stability test.All these different formulations were screened for anti-fungal activity against *Candida albicans.* The results of this study reveal that, the evaluation parametersand stability studies for all the four formulations were observed and found to be satisfactory. The F4 formulation exhibited highest inhibitory effect against *C. albicans*.Whereas F3 and F2 formulations showed similar or moderate inhibitory activity. This is the first report on the scientific evaluation of *Thuja orientalis, Punica granatum, Aloe barbadensis miller* and Coconut oil combinations as polyherbalgel formulations for anti-fungal activity as vaginal-care.

Key words: *Thuja orientalis; Punica granatum; Aloe barbadensis miller; Coconut oil; Candida albicans; Anti-fungal.*

1. Introduction:

In India, there are around 10 million cases of Vaginitis are reported annually (Maedeh Rezghi *et al*., 2019). After bacterial vaginal infections, vaginal candidiasis is the second most frequent kind of vaginal infection in the US. In the US, there are thought to be 1.4 million outpatient visits per year for vaginal candidiasis. In medicine, a variety of conditions that result in vaginal inflammation or infection are referred to as vaginitis resulted as bacteria, viruses, fungus infections. All of the conditions that result in infections and inflammation because of fungi, bacteria, yeast, viruses, and prion are collectively referred to as vargi Vaginitis is nothing but the various disorders that causes infections and inflammation due to fungus, bacteria, yeast, virus and prion (Manisha Kumari, Md. Anzer Alam. 2024) The symptoms of vaginitis that have been reported include burning, redness, itching, swelling, irritation, inflammation, and a fishy smell. Candida or yeast vaginitis, bacterial vaginitis, trichomoniasis vaginitis, chlamydia or gonorrhoea vaginitis, viral vaginitis, non-infectious vaginitis, and atrophic vaginitis are the most prevalent kinds of vaginitis. This illness affects about 90% of affected women and is secondary to either bacterial, candidiasis, or trichomoniasis. A specific kind of yeast known as Candida is the cause of a vaginal yeast infection, a kind of vaginitis. You will always have yeast in your body, but if it grows quickly, you run the risk of developing a serious illness. You may also get a vaginal yeast infection multiple times in your lifetime. There are no issues when this yeast is in balance with your body's eco-system, but when that equilibrium is thrown off, the yeast multiplies quickly and you may have a yeast infection. It is not possible to die from vaginal infections. Other names for vaginal infections are vaginal candidiasis or vulvovaginal candidiasis. In actuality, a vaginal yeast infection is a form of vaginitis, a painful, swelling vaginal discharge syndrome. While there are various forms of vaginitis, all of them have comparable symptoms, vaginal yeast infections are among the most prevalent. Candida can grow when the chemical equilibrium in your vagina is disrupted. When using medication for urinary tract infection (UTI), which is an antibiotic. The vagina's beneficial bacteria are eliminated during the course of treatment. This beneficial bacterium was in charge of controlling yeast. Without it, an imbalance results in a yeast infection. both when taking hormonal birth control (birth control) (Saranya Surendran *et al.,* 2023). Pregnancy-related hormonal shifts that produce drastic alterations. Your hormone shift may throw off the balance of Candida in your vagina. When you have diabetes, your urine contains an excessive amount of sugar, which affects the vagina. having a compromised defence mechanism. Your immune system may be suppressed by your drugs if you have HIV or AIDS. The primary causes of

virginalist's include fungi, bacteria, yeast, viruses, and prion. Also, there is great interest in Plant-derived extracts and their isolated phytochemicals as they gained increasing importance in the past few years and are also an emerging field of research for the treatment of gynaecological diseases from time of immemorial (Abbott J.1995). Anti-Candida effects have been of great interest in a range of antimicrobials over the past two decades. Candida, a fungus often associated with gynaecological infections (Dobreyski RV *et al.,* 2004). There are currently no herbal treatments on the market for the treatment of vaginitis; only synthetically marketed products are available. In order to treat an infection without using synthetic antibiotics, the best natural antibiotics that are currently available are those found in natural remedies for vaginitis, such as garlic, honey, cassia Tora, lemon balm, coconut oil, pomegranate peel, Thuja, Aloe vera, Ginger, Yogurt, Neem, Lavender oil, Curcumin, Tea tree oil, Rose petals, Mug wort, *Echinacea, goldenseal*, Clove, Oregano, etc. (Jovanni Neblett-Blackmon. 2022). Overall, usage of natural medications has been achieved most popularity due to ease of access to raw materials, the cost-effectiveness, and the scarcity of reported adverse reactions.

Also, there is great interest in Plant-derived extracts and their isolated phytochemicals as they gained increasing importance in the past few years and are also an emerging field of research. Anti-Candida effects have been of great interest in a range of antimicrobials over the past two decades. Candida, a fungus often associated with gynaecological infections. This was motivated by the rising popularity towards natural and herbal medications, due to ease of access to raw materials, the cost-effectiveness, and the scarcity of reported adverse reactions. We investigated and evaluated the anti-fungal potential of *Punica granatum, Aloe barbadensis*, Coconut oil and *Thuja orientalis* by incorporating them into a polyherbal vaginal gel and assessing its anti-fungal activity. An attempt will be made to determine whether combining the extracts will have synergistic effects.

In the view of this, the current investigation was carried out with the aim to Formulate and Evaluate Polyherbal Gel for Vaginal-Care (Vaginal Care). The prepared four different batches of this formulations were prepared and then each batch was individually screened for Antifungal activity.

2. Materials And Methods:

2.1 Collection of Plant Material:

Collection and authentication of fruit peel of *Punica Granatum*, leaves of *Thuja orientalis* and leaves of *Aloe barbadensis*. were authenticated by **Dr. N. M. Ganesh Babu,** Associate professor, Heading center of Herbal Gardens. No. 74/2, Jarakabande Kaval, Post Attur, Yelahanka, Bengaluru. 560064. Voucher specimens (PG/2022-23/0035, AB/2022-23/0036, To/2022-23/0037) are kept at the **Acharya & BM Reddy College of Pharmacy**, Soladevanahalli, Bengaluru. India.

2.2 Preparation of ethanolic extract of *Punica Granatum*:

Preparation of ethanolic extract of *Punica Granatum*: At first, weigh the 100 g of PPP powder and extracted with Methanol (70 %) by Soxhlet apparatus for 6 h. After completion of extraction process, the excess of solvent was distilled off. Collected the concentrated extract and calculated the percentage yield[7].

2.3 Preparation of ethanolic extract of *Thuja orientalis*:

At first the fresh *Thuja orientalis* leaves were collected, washed and subjected for shade drying. The dried leaves were powdered and extracted with methanol by cold maceration process. After completion of extraction, the extracts were filtered and filtrate was collected. Concentrated the filtrate to get semi solid mass and calculated the Percentage yield (Mahendra AG and Rasika DB 2019).

2.4 Preparation of ethanolic extract of *Aloe barbadensis* miller:

Fresh *Aloe barbadensis* miller leaves were collected and then scraped out with the help of clean spatula carefully avoiding the green leafy part structures. Then it was homogenized to obtain a clear homogenous mixture of the aloe gel (Mulik M and Phale M. 2009) and calculated the percentage yield. The total percentage yield of all the extracts were recorded.

2.5 Phytochemical screening of different herbal extracts:

The phytochemical screening (Tsiri *et al.,* 2009) were carried out for the both petroleum ether and alcoholic extracts of kernel seed of *Mangifera indica* and the results were depicted (Al-Zoreky NS 2009).

2.6 Preparation of Polyherbal Gel

The gel was prepared using the dried ethanolic extract of *Punica Granatum*, *Thuja orientalis*, *Aloe barbadensis* and coconut oil using Carbopol-940 (1%) as a gelling agent. Gels of individual plant extracts as well as polyherbal gels were prepared. Diclofenac sodium gel was considered as standard.

2.7 Preparation of Polyherbal gel formulations

Four different polyherbal gel formulation were prepared by simple gel preparation method with Carbopol gel base Table 1. Then Carbopol 934 of 0.75 g measured and was added slowly to the beaker. Containing above liquid while stirring. Simultaneously, added 60 ml of distilled water in the interval of 10 min. Mixed continuously by using Magnetic stirrer, kept aside for 1 h. The above solution and stirred continuously for 40 min. Further the above solution was homogenized (6- 10) minutes or until the solution was observed uniformity. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. Then, the drug extracts were added in each four formulations (F1, F2, F3 and F4) of the concentration of 0.1g,0.2g, 0.3g, 0.4g respectively followed with continuous stirring Table 2. Further, the obtained gel containing herbal extract was homogenized again (6-10) min to get the good consistency and to avoid the grittiness and polyherbal gel prepared (Umadevi A . *et al*., 2018).

2.8 Evaluation Parameters of Polyherbal Gel Formulations

2.8.1 Physical evaluation parameters:

Physical parameters such as colour, **consistency, homogeneity, grittiness** was recorded manually (Mahendra AG and Rasika DB. 2015).

2.8.2 Determination of Homogeneity:

All developed gels were packed in the containers and then tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates was recorded**.**

2.8.3 Grittiness:

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope and recorded the same.

2.8.4 Determination of Extrudability:

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked and recorded (Ambala and Vemula. 2015).

2.8.5 Determination of Spreadbility:

500mg of the cream was sandwiched between 2 slides. A weight of 100 gm was placed on upper slide. The weight was removed and extra formulation was scrapped off. The lower slide was fixed on board of apparatus and upper slide was fixed with nonflexible string on which 20g load was applied. Time taken by upper slide to slip off was recorded (Sumeet Dwivedi *et al.,* 2023).

2.8.6 Stability studies:

The stability studies for the four formulations were accessed using ICH Guidelines. The different polyherbal gel formulations (F1, F2, F3, & F4) were evaluated for them Thermostability by keeping them in the stability chamber 40 ± 2˚C (Nearly 2 months) (Saurabh Dilip Bhandare. 2009).

2.8.7 *Determination of pH*:

The pH of the herbal gel was determined by digital pH meter. One g of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained and constant reading was noted. The measurements of pH of each formulation were done in triplicate and average values are calculated (Shivhare UD *et al*., 2009).

2.9 Screening of Anti-Fungal Activity: by Cup Plate Method.

The effectiveness of produced emulgel against fungi was tested using the agar-well diffusion technique. Commercial clotrimazole cream as an antifungal standard and positive control, its topical antifungal gel mainly used to treat fungal infections like, sweat rash, diaper rash, ringworm, vaginal thrush **(**Deshmukh M. 2022**).**

The fungal strain **of** standard *Candida albicans* cultures was provided by **Acharya & BM Reddy College of Pharmacy, Department of Microbiology, Bengaluru.** A gel borer was used to hole four wells on an agar plate. The fungal strain was uniformly distributed throughout the agar bed. Using a micropipette, we put emulgel samples into four of the wells above the

agar bed and commercial clotrimazole cream into the five well. The different values of the inhibition zone have been recorded and assessed after 24 h of incubation at 37 ºC (Manjunath, K. 2014).

3. Results:

3.1 Percentage yield of extracts:

The percentage yield of three different extracts such as *Punica granatum, Thuja orientalis* and *Aloe barbadensis* miller were found to be 27 %, 57 % and 86 % respectively Table 3.

3.2 Preliminary Phytochemical screening

The results of phytochemical screening for all the extracts reveals that, the presence of alkaloids, flavonoids, tannins and phenolic compounds Table 4**.**

3.3 Physical evaluation parameters

Physical parameters such as colour, consistency, Homogeneity and Grittiness was recorded manually Table 5.

3.4 Extrudability

The percent of the extruded gel was calculated Table 6 (>90 % extrudability: Excellent, >80 % extrudability: Good, and >70 % extrudability: Fair).

3.5 Spreadbility

Time taken by upper slide to slip off was noted down **and results were** shown in Table7**.**

3.6 Stability studies

These formulations maintained by keeping them in the stability chamber 40 ± 2˚C (Nearly 2 months) Table 8.

3.7 pH Determination

The measurements of pH of each formulation were done in triplicate and average values are calculated Table 9.

3.8 Screening of Anti-Fungal Activity: by Cup Plate Method.

The anti-fungal activity of polyherbal gel F4 formulation showed highest inhibitoryeffect against *C. albicans* by cup plate method, Whereas, F3 and F2 formulation revealed similar or moderate inhibitory activity and F1 formulations shown less significant activity Table 10.

4. Discussion:

The antifungal properties of all formulations may be due to the presence of secondary metabolites like flavonoids, glycosides, tannins, triterpenoids and alkaloids. Further it is necessary to carry out further studies to confirm the role of each secondary metabolites for its antifungal activity. The antifungal activity may be less significant with a single usage of herb whereas the incorporation of these selected herbs in the gel formulation especially F4 exhibited significant antifungal activity, other formulations like F2 and F3 shown moderate activity which was reported for the first time. Thus, our study reveals that, the polyherbal gel formulation can show better healing properties for the vaginitis**.**

From the results obtained it was concluded that the poly herbal gel containing different extracts *Punica granatum, Thuja orientalis, coconut oil and Aloe barbadensis* have optimum anti-fungal activity and may be used for the treatment of gynaecological disorders. Moreover, detailed pharmacological screening and clinical approaches need to establish for the formulation of safe and effective drugs. The formulation code F4 has promising and effective drug content and release. Hence, it was concluded from the present investigation that the selected herbal formulation i.e., herbal gel for Vaginal-care (F5) have a prominent effect in the treatment of *vaginal candidiasis*, though the detailed clinical approaches need to establish for the formulated herbal gel in order to establish its of safety and effectiveness.

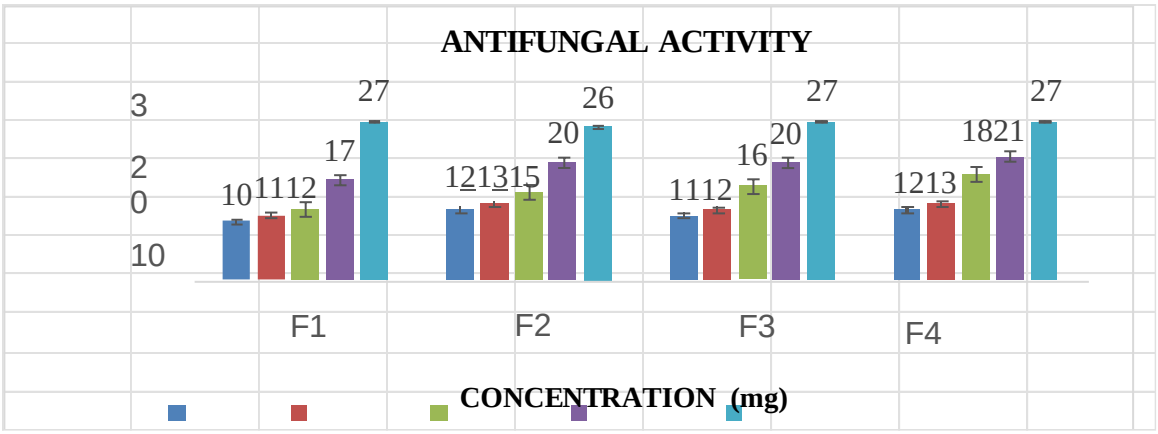

Figure 1: Antifungal Activity for all herbal formulations of standard drugs against fungal test organism.

Figure 2: Zone of inhibition of F1 and F2 formulations.

Figure 3: Zone of inhibition of F3 and F4 formulations.

Table 1: Composition of Gel Base

Sl No.	**Ingredients**	**Quantity (g)**
1	*Carbopol 934*	0.75
2	*Methyl paraben*	0.08
3	Distilled water	50ml
4	Triethanolamine	QS

Table 2: Composition of different formulations (F1-F4)

Sl No.	**Ingredients**	**F1(g)**	**F2(g)**	**F3(g)**	**F4(g)**
1	*Punica granatum*	0.2	0.3	0.2	0.4
2	*Thuja orientalis*	0.4	0.1	0.1	0.1
3	Coconut Oil	0.2	0.2	0.3	0.3
4	*Aloe barbadensis* miller	0.2	0.4	0.4	0.3
5	Gel Base	QS to 50 G	QS to 50 G	QS to 50 G	QS to 50 G

Table 3: % yield of three extracts

Sl. No	**Extracts**	**Percentage yield (%)**
1	*Punica granatum*	27.42
2	*Thuja orientalis*	57.05
3	*Aloe barbadensis miller*	86.7

Table 4: Preliminary Phytochemical screening of Different extracts

Tests	***Punica Granatum* extract**	***Thuja orientalis* extract**	***Aloe barbadensis* extract**
Alkaloids			
a. Mayer's test	-ve	+ve	-ve
b. Dragonroot's test	-ve	+ve	-ve
Steroids			
a. Salkowski test	-ve	-ve	-ve

b. Lieberman Burchard test	-ve	-ve	-ve
Coumarins			
a.Florescence response test	-ve	-ve	-ve
Carbohydrates			
a. Molisch's test	-ve	-ve	-ve
b. Benidict's test	-ve	-ve	-ve
Flavonoids			
a. Foam tests (Saponins)	+ve	-ve	+ve
b. Shinoda test	+ve	-ve	+ve
Cardiac glycosides			
a. Baljet test	-ve	-ve	-ve
b. Killer-kiliani test	-ve	-ve	-ve
Phenolic Compounds and Tannins			
a. Neutral $FeCl_3$ test	+ve	+ve	+ve
b. Dil. Iodine test	+ve	+ve	+ve
c. Dil. HNO_3 test	+ve	+ve	+ve
d. Dil. NH_4OH test	+ve	+ve	+ve
e. Acetic acid test	+ve	+ve	+ve

Note: - '-'Negative '+' Positive

Table 5: Physical evaluation like colour consistency, homogeneity, grittiness for all the Four formulations.

Formulations	**Colour**	**Phase separation**	**Consistency**	**Homogeneity**	**Grittiness**
F1	Pale Yellow	None	+	++	++
F2	Pale Yellow	None	++	+++	+++
F3	Pale Yellow	None	+	++	++
F4	Pale Yellow	None	++	+++	+++

Note: + fair, ++ good, +++ excellent

Table 6: Extrudability of all the four different herbal extracts gel formulations

Formulations	**Weight of empty tubes (g)**	**Weight of filled tubes (g)**	**Formulation filled in the tube (g)**	**Quantity Extruded**	**Extrudability (%)**
F1	3.75	26.44	25.67	18.52	Fair
F2	3.80	25.02	21.22	20.74	Excellent
F3	3.68	24.99	21.31	19.74	Good
F4	3.75	28.20	24.45	23.45	Excellent

Table 7: Spreadbility of Four different gel formulations

Formulations	**Weight tied**	**Length of slide (cm)**	**Time taken (sec)**	**Spreadbility (g x cm/sec)**
F1	10	7.5	7.2	10.41 ± 0.02
F2	10	7.5	6.0	12.50 ± 0.01
F3	10	7.5	5.7	13.16 ± 0.01
F4	10	7.5	4.0	18.75 ± 0.03

Table 8: Physical stability studies of prepared polyherbal formulated gel

Duration	**Storage Condition**	**Appearance**	**Colour**	**Odour**
10 days	8 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic
	40 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic
30 days	8 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic
	40 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic
60 days	8 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic

	40 °C	Semi-solid glossy gel	Pale Yellow	Bland, Characteristic

Table 9: pH Determination for all the four formulations

Formulations	pH
F1	4
F2	4.1
F3	4.3
F4	4.4

Table 10: Zone of Inhibition of Antifungal Activity for all herbal formulations

Formulations	Zone of Inhibition (mm) for Different Concentration of Gel Formulation (mg)				Standard (50 mg)
	5 mg	10 mg	30mg	50 mg	
F1	10 ± 0.02	11 ± 0.03	12 ± 0.02	17 ±0.02	27 ± 0.03**
F2	12 ±0.03	13 ± 0.02	15 ± 0.01*	20 ±0.04*	26 ± 0.02**
F3	11 ±0.02	12 ± 0.01	16 ± 0.02*	20 ±0.02*	27 ± 0.03**
F4	12 ±0.03	13 ±0.02*	18 ± 0.03*	26 ± 0.03**	27 ± 0.03**

*P>0.5, **P>0.01

Conclusion:

Polyherbal gel formulation was prepared, the physicochemical parameters and stability studies for all the four formulations were observed and found to be satisfactory. The antifungal properties of all formulations may be due to the presence of secondary metabolites like flavonoids, glycosides, tannins, triterpenoids and alkaloids.

It is necessary to carry out further studies to confirm the role of each secondary metabolites for its antifungal activity. The activity may be less significant with a single usage of herb whereas the incorporation of these selected herbs in the gel formulations exhibited significant antifungal activity, reported for the first time.

Thus, our study reveals that, the polyherbal gel formulation can show better healing properties for the vaginitis.

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Author contribution statement

Hemalatha. K: Supervision, Formal analysis and writing the original draft. J Joysa Ruby: Writing and review and editing, Data curation. Venkatesh DP: validation and supervision, conceptualization, N Athmika: and Miriyam Claudish: Evaluation parameters for formulations.

Conflict of interest statement

On behalf of co-authors, I declared that we do not have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference:

Abbott J. (1995). Clinical and microscopic diagnosis of vaginal yeast infection: a prospective analysis. Annals of emergency medicine. 25(5), 587- 951.

Ali U, Kumar P. (2014). Effect of Soxhlet and ultrasound assisted extraction on antioxidant activity of pomegranate peel extract. International Journal of Food and Nutritional Sciences. 3(6), 265. http:/www.ijfans.com/currentissue.html

Al-Zoreky NS. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International journal of food microbiology.134(3), 244-248. 10.1016/j. ijfoodmicro.2009.07.002

Ambala and Vemula, (2015) Ambala, R, and Vemula, S. Formulation and characterization of ketoprofen emulgels. Journal of Applied pharmacy and Science. 5(7), 112-117.10.7324/JAPS.2015.50717

Deshmukh, M.; Makde, P.; Baheti, J. and Thote, L (2022). Green synthesis of zinc oxide nanoparticles (Zno-Nps) Ailanthus excelsa roxb. stem bark extract and its antibacterial activity. Ann. Phytomed. 11(2):743-747**.** 10.54085/ap.2022.11.2.90

Dobrevski R. V., Kovachev S., Nacheva A., Stoev S and Vasilev N. (2004). Comparative study of itraconazole and fluconazole therapy in vaginal *candidiasis*, Akush Ginekol. Europe PMC. 43(1), 20-23. 10.1016/j.ajog.2007.10.786

Jovanni Neblett-Blackmon. (2022). Home Remedies for Bacterial Vaginosis. Healthline. 22: www.healthline.com/health/home-remedies-forbacterial-vaginosis.

Maedeh Rezghi, Homa Hajimehdipoor, Sara Zakerin, Shirin Fahimi. (2019). Preparation of traditional poly Herbal Gel for Abnormal Vaginal discharge. Research. J Pharmacog. 6(4), 27-35. 10.22127/rjp.2019.93506

Mahendra AG and Rasika DB. (2015). Formulation and evaluation of topical anti-inflammatory herbal gel. Asian Journal of Pharmacy and Clinical Research. 12, 252-255. 10.22159/ajpcr. 2019.v12i7 .33859

Mahendra AG, Rasika DB. (2019). Formulation and evaluation of topical anti- inflammatory herbal gel. Asian J Pharm Clin Res. 12, 252-255. http://dx.doi.org/10.22159/ajpcr.2019.v12i7.33859

Manisha Kumari, Md. Anzer Alam (2024). Flowers Used for Treatment of Gynaecological Disorders by Women of Saran District. Int J Sci Res. 13(1), 974-976. https://dx.doi.org/10.21275/SR24115183831

Mulik M and Phale M (2009). Extraction, purification and identification of aloe gel from Aloe vera (*Aloe barbadensis* Miller). Journal of Natural Product. 5(3), 111-115.

Ravishankar, T.; Kumar, D.; Priyanka, K.; Varghese, T. Naika, H (2014). Facile combustion synthesis of ZnO nanoparticles using *Cajanus cajan* (L.) and its multidisciplinary applications. Mater. Res. Bull.1(57), 325-334. https://doi.org/10.1016/j.materresbull.2014.06.010

Saranya Surendran, Priya Prasannan, Yasotha Jeyaram, Venkatesh Palanive, Arjun Pandian, Raju Ramasubbu. (2023). Knowledge on ethno-gynaecology of Indian Tribes- a comprehensive review. J Ethnopharmacol. 303(1), 115880. https://doi.org/10.1016/j.jep.2022.115880

Saurabh Dilip Bhandare, Pravin Arungiri Gosavi and Vijay Dhondiram Wagh. (2024). Formulation and evaluation of polyherbal gel. World Journal of Pharmacy and Pharmaceutical Sciences. 13(3), 116-146.**10.20959/wjpps20243-26736**

Shivhare U.D., Jain K.B., Mathur V.B., Bhusari K.P., Roy A.A. Sharad (2009). Formulation development and evaluation of diclofenac sodium gel using water soluble polyacrylamide polymer. Digest Journal of Nanomaterials and Biostructures. 4, 285-290. https://www.researchgate.net/publication/237823074

Sumeet Dwivedi, Vishal Satish Patil, Jyoti Saxena, Amit Gangwal, Deepak Shrivastava and Mahavir Chhajed. (2023). Formulation and Evaluation of Herbal cream for the treatment of *Vaginal Candidiasis*. Section A-Research paper. European Chemical Bulletein.12(1), 586-593. https://www.researchgate.net/publication/371044174

Tsiri D, Graikou K, Pobłocka-Olech L, Krauze-Baranowska M, Spyropoulos C, Chinou I (2009); Chemo systematic value of the essential oil composition of Thuja species cultivated in Poland—antimicrobial activity. Molecules. 14(11), 707-4715. **https://doi.org/10.3390/molecules14114707**

Umadevi A, Kumari C, Kumar PA, Am HS, Divya K, Hisana PV. (2018). Development and evaluation of polyherbal gel for antifungal activity. International Journal of current pharmaceutical Research. 10(5), 40-43. DOI:10.22159/ijcpr.2018v10i5.29694