International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 248-258 www.ijprajournal.com ISSN: 2456-4494

Formulation Development and Evaluation of Polyherbal Antifungal Shampoo

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Submitted: 05-09-2022 Accepted: 13-09-2022

ABSTRACT:
The present work labels a study on formulation design and evaluation of Polyherbal antifungal shampoo. This study consists of essential oils of garlic, Neem, oregano, cinnamon bark as prominent agents for anti-Candida or antifungal activity. These essential oils were selected for study because they appear to be more prominent as antiviral activity and tolerated well. Combination of these essential oils was used to produce synergistic effects in counter to Candida Albicans. Several Excipients such as quid paraffin or as carrier of essential oils, beeswax as base and borax as emulsifying agent were used to produce significant formulation in the form of shampoo. The shampoo were prepared by using 2.5% and 5% combination of essential oils respectively have their own acceptable results as demonstrated in the experimental study. The pH of formulations was 6.4 to 6.6 and viscosity as per Brook field viscometer 11 was in the range of 1445-1552. Shampoo was physically evaluated and found no aggregations results of which showed stable homogeneity with excellent consistency. In terms of spredibility the formulation containing 5% combination of essential oils has more value (15) that both containing 2.5% essential oil and herbal marketed antifungal shampoo. In contrast to hair irritancy it was experienced that there was no evidence of sins type of allergic reactions or redness or irritancy by designed antifungal formulations. Stabilities parameter inclusion of accelerated stability as per standard procedure and on standard value in terms of temperature and humidity was of significant results. Micro-biological studies were carried out to determine antifungal testing of both formulations of consideration of zone of inhibition and minimum inhibitory concentration and it was found that shampoo containing 9% combination of essential oil had more anti-candida activity (ZOI is 50mm) than shampoo containing 2.5%, combination of essential oil (20146mm) and herbal marketed antifungal shampoo (ZOI 17mm). In contrast of MIC also shampoo having 5% EOs was of magnificent nature in comparison of both shampoo have 2.5% EOs and marketed herbal antifungal shampoo respectively. From this investigation, it was concluded that both formulations has produced magnificent activity against fungal agents (candida albicans in this experimental study) but shampoo containing 5% EOS has more prominent then other one and also than herbal marketed antifungal formulation, Grounded on the results from the experimental study further usefulness of the dosage form might depend on pharmacokinetic data. Forthcoming or subsequent research work of antiviral activity of these essential oils may contribute in the challenging areas.

Keywords: Antiviral Activity, Anti-candida Activity, Polyherbal Antifungal Shampoo

I. INTRODUCTION:

Fungal Infection:

Fungal infection usually called mycosis is a disease which caused by a fungal agent. Millions of species of fungi are exist in environment which lives in many areas such as dirt, plant, household surface etc. Usually it begins with as a small sore, scaly hair or rashes and at very severe condition of ten spread to whole body as itches and burns.

Fungal infections are classified into following groups.

Superficial Infection:

Pathogenic fungi caused infections which are often restricted to the human hair, nails, epidermis, and mucosa and demonstrated as superficial fungal infections. Till now three most basic sorts of superficial fungal infections have been demonstrated are Dermatophytosis, pityriasis vesicular and Candidiasis.

Cutaneous Infection:

Noninvasive infections of the hair or nails are cutaneous fungal infections that make specific pathologic changes in the host cell. The fungi used infections such as Candidiasis can be yeast-like and cause tinca infections can be mold-like. Are usually treated with topical medicinal agents are usually use to treat cutaneous infections, but treated oral medicinal antifungal agents are frequently used to

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treat persistent and non-responsive infections in better way. Cutaneous Candidiasis is a most common hair infection that occurs in moist, creased, and warm areas of the body. Its incidence increases among people who have diabetes, are obese, are taking antibiotics and oral contraceptives, or are HIV positive. Candida species mainly cause cutaneous fungal infection.

Subcutaneous Infection:

In subcutaneous mycoses dermis and subcutaneous tissues of hair are mainly involve and rarely disseminate or penetrate into systemic circulation and create a severe disease Incidence of infection involves when insertion of pervasive organisms into the hair the local trauma carried out and are typically found in zones of tropical. Similarly to other mycoses, patients who are immunosuppressed or those have low immunity, also at exponentially amplified risk for these kinds of infections Sporotrichosis, Mycetoma, and Chromo blast mycosis are considered as prominent type of subcutaneous mycosis.

Systemic Infection:

Resourceful fungal pathogens caused systemic infection to which immune compromised hosts are prominently susceptible. Though, if the dose of exposure is extreme high or alongside primary (dimorphic) fungal pathogens then consequences of which even immunocompetent can he able to catch the invasive diseases As consequences of inhalation species such aspergillus, Cryptococcus, mucorales cause systemic infection typically in lungs or as consequences of infected line or breakage from GIT species such as candida may cause systemic infection and spread to other organs as well.

Opportunistic Infection:

Those individuals who are taking immunosuppressive medication and infected with pulmonary diseases or triggered by diabetes are at an exponentially augmented risk of infection acquired environmentally alongside opportunistic fungal infections. In severely immuno compromised opportunistic invasive pulmonary or sinus infection oftenly caused by Aspergillus.

Table.1 Classification of Fungi by their Disease Manifestation

Clinical manifestation	**Examples of Fungal Infection.**
Superficial Mycosis	Malassezia Infection
Cutaneous Mycosin	Candidiasis and Dermatophytos
Subcutaneous Mycosis	Sporotrichosis and Zygomycosts
Systemic Mycosis	Blastomycosis and Histoplasmosis
Opportunistic Mysicais	Aspergillosis and Cryptococensis

Table.2 Candidal & Non-Candidal Fungal Infections and Etiologies

Infection	**Etiological Agents**
Candidiasis	Candida albicans and various strains
Aspergillosis	Aspergillus fumigates
Cryptococccosis	Cryptococcus neoformans
Histoplasmosis	Histoplasma capalotum
Blastomycosis	Blastomyces dermatitidis
Zygomycosis	Order micorales and entomophthorales
Coccidio mycosis	Coccidioides immitis
Paracoccidiomycosis	Paracoccidioides brasiliensis
Sporotrichosis	Sporothrix schenckii

Experimental Work

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Volume 7, Issue 5 Sep-Oct 2022, pp: 248-258 www.ijprajournal.com ISSN: 2456-4494

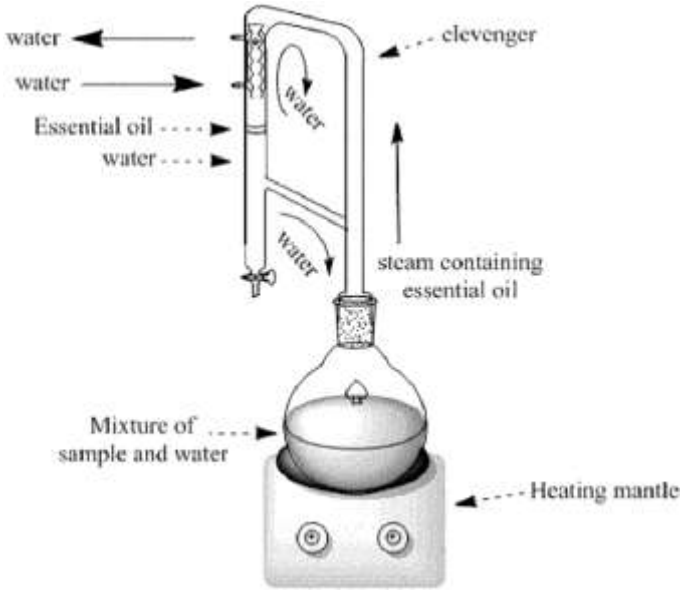

Table.3 Material Used in Research Work

S. No.	Material Used	Source
1	Garlic oil	Moksha Lifestyle Products
2	Neem oil	Moksha Lifestyle Products
3	Oregano oil	Moksha Lifestyle Products
4	Cinnamon bark oil	Moksha Lifestyle Products
5	Olive oil	Moksha Lifestyle Products
6	White bees wax	College laboratories
7	Liquid paraffin	College laboratories
8	Borax	College laboratories
9	Methyl Paraben	College laboratories
10	Propyl Paraben	College laboratories

Table.4 Instruments Used in Research Work

S. No.	Instruments
1	Magnetic stirrer
2	pH meter
3	Digital balance
4	Heating plate
5	Viscometer
6	Oven
7	Incubator

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Formulation:

Herbal antifungal shampoo of different concentration was formulated using essential oils of garlic, Neem, Oregano and Cinnamon bark alongside using Excipients. Different Excipients of different concentrations were involved to design the formulation. After initial trials the Excipients that give product of good quality and good consistency. The concentrations of essential oils were different in both formulations.

Fig.1 Procured Essential oils

Table.5 Formulation (F) Ingredients of Polyherbal shampoo containing 2.5% essential oil.

	Ingredients	**Concentration (% W/W)**
Oil phase	White beeswax	17.00
	Liquid paraffin	37.00
	Garlic oil	0.62
	Neem oil	0.62
	Cinnamon bark oil	0.62
	Olive oil	0.62
	Oregano oil	8.00
Aqueous phase	Borax	1.00
	Methyl Paraben	0.02
	Propyl Paraben	0.02
	H2O	Qs to 100%

Preparation of Polyherbal Shampoo Containing 2.5% Essential Oils:

Procedure:

- The ingredients were accurately measured or weighed as per the table.
- The water soluble components which come under aqueous phase were heated on water

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bath shaker at 75 ± 1^0 Celsius in separate beaker.

- Oil soluble components which come under oil phase were also heated at $75\pm1°$ Celsus in separate beaker followed by addition of essential oils (concentration 2.5%) in phase under constant stirring.
- Then to the heated aqueous mixture, oily phase was incorporated with incessant stirring on magnetic stirrer up to the emulsion cooled down.

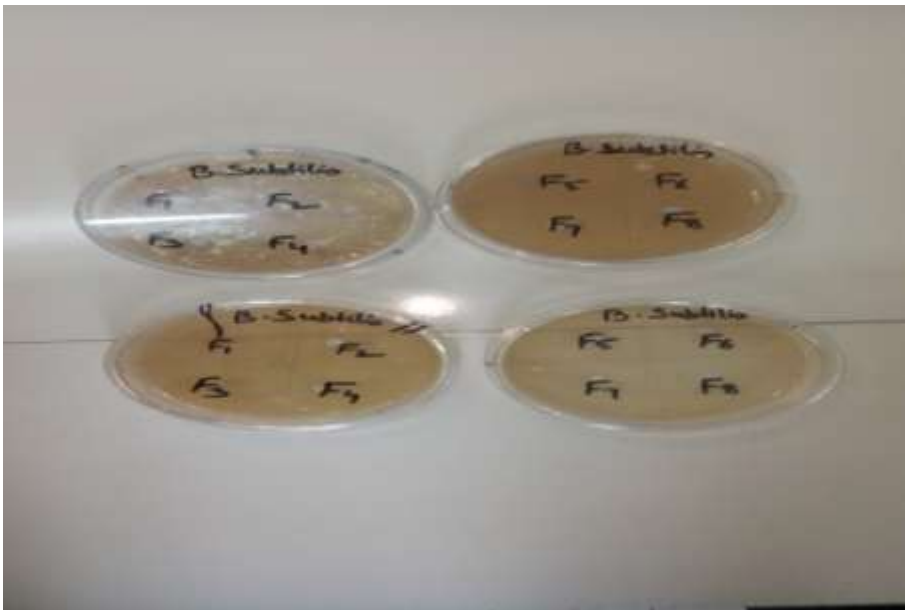

Fig.2 Prepared Polyherbal Antifungal Shampoo

In Vitro Evaluation of Polyherbal Antifungal Shampoo:

pH Determination:

pH of shampoo of various concentrations was measured by a digital pH meter calibrated bath std. buffer solution having pH 4.0 and pH 7.0. about 5 gm, of shampoo was weighed and transferred into 100 ml beaker into which 45 ml of detailed water was transferred and pH was determine at 25℃ temperature.

Table.6 pH of Polyherbal Antifungal Shampoo

FORMULATION CODE	**pH (at 25℃)**
FI	6.4
F2	6.6

Determination of Homogeneity:

Bath developed formulation was checked by visual inspection for homogeneity after the cream was put in the jar. They were checked for the existence of any aggregates and their appearance.

Viscosity:

In order to determine the viscosity values, viscosity measurements were performed by using rotational viscometer DV-III Ultra (Brook field engineering laboratories) Speed of rotation at 20-RPM and measurements were perform at 22℃ temperature by using spindle 64.

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Table.7 Viscosity of Polyherbal Antifungal shampoo

FORMULATION CODE	Viscosity (CP) at 20-RPM
F1	14589
F2	15680

Spread Ability Studies:

A significant criterion for semisolids is that they have a strong propagation power. It is a concept expressed to represent the degree to which, when applied to the hair, the shampoo easily spreads the therapeutic effectiveness of the semisolid topical formulation also depends in a large extended its spread ability benefit. The basic apparatus was designed to assess semi-solids formulations spread ability, however. Spread ability is often defined as the time taken m sec. by two sides to slip away from formulation that is put amid two slides under the application of a definite load. It has been shown that the shorter the time needed to separate the two glass slides the better the spread ability or spread value. Two glass slides of normal & defined dimensions were chosen, and over any each of the slides was placed the prepared formulation whose spread ability had to be calculated. Then the other one was put on uppermost surface of the formulations that were sandwiched along the slide between the two slides that were 5 en long By putting a weight of 100 gm on the upper slide, a uniform thin layer of antifungal shampoo (semi-solid formulation b/w, two slides) was obtained The weight was then stripped and the surplus of shampoo clinging the slides was scrapped off One of the glass slides on which the shampoo was mounted was fixed and the second movable glass slide was positioned over it. A string to which specified load could be applied using a simple pulley and a pan was connected to the one end of the second movable slide. A weight of 30gm was placed on the pan and the time taken to travel the distance of 5.0cm for the upper movable slide and detach it from the lower one slide under the direction of the weight applied was noted.

The formula given the spread value was then determined.

Spread ability =m×l/t

Where,

M = Weight tied to the upper slide (30g)
L = Length of glass slide (5cm)
T = Time taken in seconds

Table.8 Spread ability of Polyherbal Antifungal shampoo

Formulation	Time (In Second)	Spread ability (g/cm)
F1	11	13.63
F2	10	15
Marketed Formulation	12	12.5

5.2.4 Self-Irritancy Patch Test:

The area of 1 square cm on the dorsal surface of left hand was marked. The shampoo was applied under a cotton patch to the stipulated area and time was noted. Irritation, erythema and edema were examined, if any, at regular intervals of up to 24 hours and recorded.

Table.9 Self-Irritancy Patch Test of Polyherbal Antifungal shampoo

Formulation	Irritancy	Erythema	Edema
F1	No	No	No
F2	No	No	No

Stability Testing:

Stability testing is one of the most important parameter which begins with discovery of drug product and ends with demise of commercial product. ICH guidelines were followed to carry outs stability testing of antifungal shampoo. The prepared shampoo was filled in sterilized container and kept in humidity chamber & maintained at 30-2℃/ 65±5% Relative Humidity & 40 ± 2^0C/75-5% Relative Humidity for three

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months. At the end samples were examined for the organoleptic characters and viscosity too.

Table.10 Stability Studies of Polyherbal Antifungal shampoo

Tests	**Formulation F1**			**Formulation F2**		
	After 4 week	**After 8 week**	**After 12 week**	**After 4 week**	**After 8 week**	**After 12 week**
Physical Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Texture	Fine	Fine	Fine	Fin	Fine	Fine
Color	Creamy White	Creamy White	Cream y white	Creamy white	Creamy White	Creamy white
Odor	Characteristi c	Characteristic	Chara cteristi c	Characteri stic	Characteris tic	Characterist ic
pH value	6.4	6.2	6.2	6.6	6.4	6.3
Thermal stability	Fine	Fine	Fine	Fine	Fine	Fine
Degradation of product	No	No	No	No	No	No
Viscosity	14450	14310	14300	15520	15430	15400

Table.11 Accelerated Stability Studies of Polyherbal Antifungal shampoo

Tests	**Formulation F1**			**Formulation F2**		
	After 4 week	**After 8 week**	**After 12 week**	**After 4 week**	**After 8 week**	**After 12 week**
Physical Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Texture	Fine	Fine	Fine	Fin	Fine	Fine
Colour	Creamy white	Yellowish White	Yellowish White	Creamy white	Yellowish White	Yellowi sh White

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Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
pH value	6.4	6.1	6.5	6.6	6.2	6.7
Thermal stability	Fine	Fine	Fine	Fine	Fine	Fine
Degradation of product	No	No	No	No	No	No
Viscosity (CPs)	14450	14010	13750	15520	15210	15130

Accelerated Stability Studies was performed for antifungal shampoo at 40 ±2℃ / 75 ± 5% Relative Humidity for three months.

Microbiological Studies:

Antifungal Testing of Shampoo:

Preparation of Media:

To assets the in vitro operation, Saboraud's Dextrose Broth was used the media has been prepared as per manufacturer's instructions. Using a weighing balance, about 19.5grams of Sabouraud Dextrose Agar (SDA) was weighed and dissolved into a conical flask containing 300 ml of distilled sterile water. Then it was shaken. Mixed and dissolved in a hot dish. The pH of same was changed as needed. The media was then placed in an autoclave for sterilization at a pressure of 15lbs at 121℃ or 15 minutes. The media was then allowed to cool down to approximately 45℃ and then poured intopetri plates which are previously sterilized. Then plates were allowed overnight to solidity. For further use, the plates were then held at 4℃

Method:

Disk Diffusion Method:

With the aid of a sterile cotton bad saturated with the culture, 0.1 ml of the freshly revived culture (24h old culture) was pipette out and spread uniformly. The place was swirled at an angle of 45 and distributed uniformly throughout. For 5 minutes the plate was held aside and. allowed to dry. Wells were produced in the center of the plate with the help of a sterile cork borer after swabbing the culture. In the well 0.1gm of the sample was then filled then plat s were incubated for 48 hours at 37℃. The inhibition zone was then calculated and reported with the aid of a standard reader for the antibiotic zone. The results well diffusion methods are as tabulated in following table.

Table.12 Zone of Inhibitions

Formulation	**Zone of Inhibition**
F1	46 mm
F2	50 mm
Herbal Marketed Formulation	17 mm

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Fig.3 Zone of Inhibition of Antifungal Shampoo against Candida Albicans.

HMF-Herbal marketed formulation.

Determination of Minimum Inhibitory Concentration (MIC):

There no apparent turbidity is observed in the test tube, MIC is classified as the lowest concentration. In this process, the technique of broth dilution was used where formulations were prepared in sterile DMSO at the maximum concentration of 50µg/ml and serially calculated to a working concentration varying from 10µg/ml to 50µg/ml using SD broth forgen and then inoculated with 40ml suspension of the test species. Test tubes were noted for Turbidity after 24 hours of incubation. The lowest concentration was determined and noted MIC if no turbidity was observed.

Table.13 Minimum Inhibitory Concentration

Formulation	MIC
F1	25µg/ml
F2	20µg/ml
Herbal Marketed Formulation	45µg/ml

II. RESULTS AND DISCUSSION

Formulation of Polyherbal Antifungal Shampoo Using Various Conc. Of Essential Oils:

Polyherbal shampoo formulations were prepared which include different essential oils and the oils are Garlic EO. Neem EO, Oregano EO and Cinnamon bark EO. These oils have been used in formulations is main active agents and apart from the olive nit also used in the formulations as carrier oil alongside several other main Excipients five used to obtain stable formulations as per procedure.

Formulation of Shampoo (F1) Using Essential Oils with 2.5% Concentration:

Shampoo was prepared according to formula table followed by referring the standard procedure and due to result of which obtained shampoo (F1) was of desired consistency.

Formulation of Shampoo (F2) Using Essential Oils with 5% Concentration:

Cream was prepared according to formula table followed by referring the standard procedure and due to result of which obtained shampoo (02) was of desired consistency.

Evaluation of Polyherbal Antifungal Shampoo:

pH Determination:

pH of prepared shampoo of different concentrations was resulted out with the help of digital pH meter. The pH of formulations was 6.4 and 6.6 respectively and the results were shown in table.6.

Determination of Homogeneity:

Formulations were tested for homogeneity and aggregation and both Formulations were found with stable homogeneity alongside no evidence of aggregation.

Viscosity:

Viscosity measurements were performed by using rotational viscometer DV-III (brook field engineering laboratories). The viscosity of both formulations was 14589 and 15680 respectively and results were shown in table.7.

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The Spread Ability Studies:
The spread ability of shampoo were determined according tasted procedure and The spread ability data showed that both formulations had The spread ability value 13.63 & 15 respectively and compared with the spread ability of herbal marketed shampoo which was 12.5. Resulted data was showed in table.8.

Self-Irritancy Patch Test:
The shampoos were applied to the specified area under a cotton patch and time was noted down. There were no evidence of any type of Irritancy, erythema and edema Apart from the farther results were showed in table.9.

Stability Testing:
Stabilities testing were performed as per procedure (at 30±2Celsius/65±5 % Relative Humidity and alongside 40 ±2Celsius75±5% Relative Humidity for two months) including Accelerated Stability testing for various parameters and both formulation stabilities parameters were in acceptable range and detailed resulted data were shown in table.10 and 11.

Microbiological Studies:
Under microbiological studies both formulations were tested for antifungal activity considering their zone of inhibition and minimum inhibitory concentration and compared with herbal marketed formulation.

Zone of Inhibition:
Saboraud's Dextrose Broth media was utilized for determining antifungal activity of both formulations against candida albicans using disk diffusion method and the zones of inhibition of FI and F2 were 46mm and 50mm respectively, alongside compared with herbal marketed formulation (shampoo) had 17mm zone of inhibition, further more results were tabulated in table.12.

Minimum Inhibitory Concentration:
Both designed formulation was evaluated for their MIC and compared with herbal marketed antifungal formulation. No turbidity was found at 25µg/ml for F1 and 20ug mi for F2 which subsequently compared with herbal marketed formulation had MIC at 40g ml further more results were demonstrated in table.13.

III. SUMMARY

The present work labels a study on formulation design and evaluation of polyherbal antifungal shampoo. This study consists of essential oils of garlic, neem, oregano, cinnamon bark as prominent agents for anti-candida or antifungal activity. These essential oils were selected for study because they appear to be more prominent as antiviral activity and tolerated well. Combination of these essential oils was used to produce synergistic effects in counter to candida albicans. Several excipients such as quid paraffin or as carrier of essential oils, beeswax as base and borax as emulsifying agent were used to produce significant formulation in the form of shampoo. The shampoo were prepared by using 2.5% and 5% combination of essential oils respectively have their own acceptable results as demonstrated in the experimental study. The pH of formulations was 6.4 to 6.6 and viscosity as per Brook field viscometer 11 was in the range of 1445-1552. Shampoo was physically evaluated and found no aggregations results of which showed stable homogeneity with excellent consistency. In terms of spredibility the formulation containing 5% combination of essential oils has more value (15) that both containing 2.5% essential oil and herbal marketed antifungal shampoo. In contrast to hair irritancy it was experienced that there was no evidence of sins type of allergic reactions or redness or irritancy by designed antifungal formulations. Stabilities parameter inclusion of accelerated stability as per standard procedure and on standard value in terms of temperature and humidity was of significant results. Micro-biological studies were carried out to determine antifungal testing of both formulations of consideration of zone of inhibition and minimum inhibitory concentration and it was found that shampoo containing 9% combination of essential oil had more anti-candida activity (ZOI is 50mm) than shampoo containing 2.5%, combination of essential oil (20146mm) and herbal marketed antifungal shampoo (ZOI 17mm). In contrast of MIC also shampoo having 5% EOs was of magnificent nature in comparison of both shampoo have 2.5% EOs and marketed herbal antifungal shampoo respectively.

IV. CONCLUSION:

From this investigation, it was concluded that both formulations has produced magnificent activity against fungal agents (candida albicans in this experimental study) but shampoo containing

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