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PHOTOCHEMICAL ANALYSIS AND ANTI-DANDRUFF ACTIVITY OF VARIOUS MEDICINAL PLANT EXTRACTS AGAINST MALASSEZIA SPPS

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ABSTRACT

This experiment was subjected to find out anti dandruff properties of fifteen different medicinal plants that are locally available in Lakhani region of Maharashtra. Flaking of the scalp is the result of seborrhea dermatitis, also called dandruff. Dandruff is a condition of the scalp that causes flakes off the skin to appear. Oily scalp, hormones or a fungus Malassezia seems to be the cause of dandruff. So, There is need to assume resourceful research into this area. The research work was divided into two parts. Part1 which demonstrate the phytochemical analysis of all the selected medicinal plants. The aqueous plant extracts of samples were used for phytochemical analysis. The phytochemical analysis of the plants is important as they have great interest in pharmaceutical companies for production of new drugs for curing of diseases and Part 2 which demonstrates the antidandruff activity of all the selected plants. Our aim was to check antidandruff activity of this fifteen medicinal plants, experiments was carried out on Malassezia spps., the causal organism for dandruff which was isolated using SDA by well diffusion method and comparision with chemical based shampoo. An attempt was made to analyze the presence or absence of different phytochemicals in all selected plants and to check out antidandruff activity. Thus, Medicinal plants have some natural antimicrobial property and therefore such combination could be a potential antidandruff activity.

KEYWORDS: Anti-dandruff activity, Medicinal plants, Malassezia spps., Photochemical analysis.

INTRODUCTION

Since ancient time medicinal plants have been discovered and used in traditional medicine preparations.[1] These medicinal plants synthesize hundreds of chemical compounds that are known as phytochemical. These chemicals are produced by plants through primary and secondary metabolism. They play a role in plants growth and/or defense against competitors, pathogens, insects or predators.[2] Some phytochemicals are used as poisons and others are used as traditional medicine.

The leaves and fruits of selected plants were washed, air dried and then powdered. The aqueous extracts of the plants and fruit samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. Phytochemicals have two categories i.e. primary and secondary constituents.[3] Primary constituents contains chlorophyll, protein, sugars. Secondary constituents contain flavonoids, alkaloids, terpenoids, tannin etc.[4]

Terpenoids exhibit various important pharmacological activities i.e., anti- inflammatory, anticancer, antimalarial, antiviral and antibacterial activities Terpenoids are very important in attracting useful mites and consume the herbivorous insects.[5] Alkaloids are used as anaesthetic agents and are found in the medicinal plants.[6]

Momordica charantia (Karela) are used for treatment of diabetic patients and also useful in lowering of blood glucose levels in blood The bioactive constituents are present in the *Momordica charantia* that is charantiosides, momordin and glucosides. It also includes terpenoids constituents such as momordin, momordenol, momordicin-28, momordicilin and momordol.[7-11] *Morus nigra (Saituk)* is binomial name of mulberry which have shown various biological properties such as anti-inflammatory activities.[12] Guava is the common name of the *Psidium guajava* whose phytostudy shown that its extracts have more than twenty compounds.[13]

Aloevera leaves contains phytochemicals such as acetylated mannans, anthraquinone, anthrone, C-glycosides and other anthraquinones like emodin and various lectins. It's extracts are largely used in beverages, skin lotions, cosmetics, oinments for minor burns and sunburn and used as medicine *Phyllanthus emblica* which known as emblic, amla or Indian gooseberry is directly used in traditional Indian medicine. It is commonly used in inks, shampoos and hair oils. It contains high amount of tannin.[14] *Mentha spicata* known as the mint or garden mint or lamb mint. It mainly contain chemical components of the terpenoids

carvonene which is antitumar in nature. It is historically used for it's antimicrobial activity due to it's high concentration of the carvonene. Tulsi whose binomial name is *Ocimum sanctum contains* some phytochemical constituents as ursolic acid, rosmanic acid, eugenol, carvacrol, linalol and mostly terpenes. The studies reveal that tulsi has unique combination of actions that includes: antibacterial, antiviral, antifungal, antimalarial, antiprotozoal, antihelmintic, mosquito repellent, antioxidants, radioprotective, neuroprotective, antidiabetic, antiasthmatic, antithyroid, antistress, memory enhancement and anticoagulant activities.

Curcuma longa (Turmeric) contains curcumin which is responsible for it's golden yellow color. It's phytochemical components includes diarylheptanoids a class includes numerous curcuminoids. *Spindus mukorassi* known as Indian soapberry, washnut or soapnut which contains compounds of saponin thus used as a cleanser for hair, skin and clothing. *Azadirachta indica* commonly known as the need or Indian lilac contains nimbin, nimbinin and nimbinin which are identified as the active antibacterial ingredients. They also serves as a natural insecticide.

Keeping in view the importance of phyto-chemicals, vitamins and other important constituents of these medicinal plants and their wide use by the local community and practitioners for a variety of ailments, it is therefore very vital to discern their active constituents and to provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals, vitamins and other constituents Keeping in view the importance of phyto-chemical vitamins and other important constituents of these medicinal plants and their wide use by the local community and practitioners for a variety of ailments, it is therefore very vital to discern their active constituents and to provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals, vitamins and other constituents Keeping in view the importance of phyto-chemicals, vitamins and other important constituents of these medicinal plants and their wide use by the local community and practitioners for a variety of ailments, it is therefore very vital to discern their active constituents and to provide a scientific data base line which may play a significant role in knowing the quantities of these phytochemicals, vitamins and other constituents.

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line which may play a significant role in knowing these phytochemicals, other constituents (Iqbal Hussain, et al. 2011).

And about anti dandruff activity, Dandruff is a common scalp condition in which small pieces of dry skin flakes off of the scalp. Dandruff also makes scalp itch. Many people believe that dandruff is caused by the poor hygiene but not all true The main cause of dandruff is seborrheic dermatitis a condition that turns the skin oily, red and scaly.[16] Often a fungus called malassezia triggers dandruff. Although it's not harmful but can be quite embarrassing and itchy. Malassezia converts the sebum lipid into fatty acids and triglycerides, which accelerate hyperproliferation of keratinocytes. Males are more affected than females by dandruff.[15] Dandruff usually begins in young adulthood and continues through middle age for some people it may a problem for lifelong .Certain diseases like Parkinson's, Alzheimer's and other diseases that affect the nervous system also seems to increase the risk of dandruff (Ranganathan and Mukhopadhay, 2010). Some shampoos have different active ingredients such as coal tar, pyrithione zinc, salicylic acids, sulphur, selenium sulfide and ketoconazole as a key ingredients.[16] These chemical treatment options have certain limitations, which may be due to poor efficacies or due to compliance issues.[17,18] The present study involves the fifteen different medicinal plants namely *Aloevera barbadensis, Phyllanthus emblica, Ocimum sanctum, Spindus mukorassi, Mentha spicata, Acacia concinna, Tamarindus indica, Curcuma longa, Vichella nilotica, Moringa oleifera, Momordica charantia, Morus nigra, Azadirachta indica, Psidium guajava, Corica papaya* that locally available in the Lakhani region of the Maharashtra (India).

This work is aimed at investigating based on effectiveness of natural plant extract containing anti dandruff activity in comparision with chemical based shampoos that are easily available in market. The main objective of this research work was to analyze the presence or absence of different phytochemicals in all fifteen selected plants and to check it out whether they show antidandruff activity or not which may used for healing and curing of various diseases.

MATERIAL AND METHOD

PART 1: Phytochemical analysis

Plant materials

The present study included the plant species which were *Aloevera barbadensis, Phyllanthus embllica, Osmium sanctum, Spindus mukorassi, Mentha spicata, Acacia concinna,*

Tamarindus indica, Curcuma longa, Vichella nilotica, Moringa oleifera, Momordica charantia, Morus nigra, Azadirachta indica, Psidium guajava and Carica papaya.

Chemicals

Fehling solution A and Fehling solution B, ethanol, distilled water, aqueous HCL, methanol, chloroform, concentrated sulphuric acid, ammonia solution, picric acid, hexane.

Sample collection

Fifteen medicinal plants were collected locally from the farm lands of Lakhani area (Maharashtra, India). The plants were used for the purpose of their phytochemical analysis. The plants collected were identified botanically in Department of Botany, Kamla Nehru Mahavidyalaya, Nagpur University, Nagpur. Fresh and tender leaves and fruits of selected plants were used for the phytochemical analysis. Plant species selected during present investigation were given in the Table no.1.

Preparation of plant extract

The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and then leaves were macerated into powder and stored in polythene bags for use.

The plant powder was taken in a test tube and add distilled water. Mixed it thoroughly then boiled it till its amount reduces a bit. The solution then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for the experiment.(Figure 1, 2).

Figure 1:

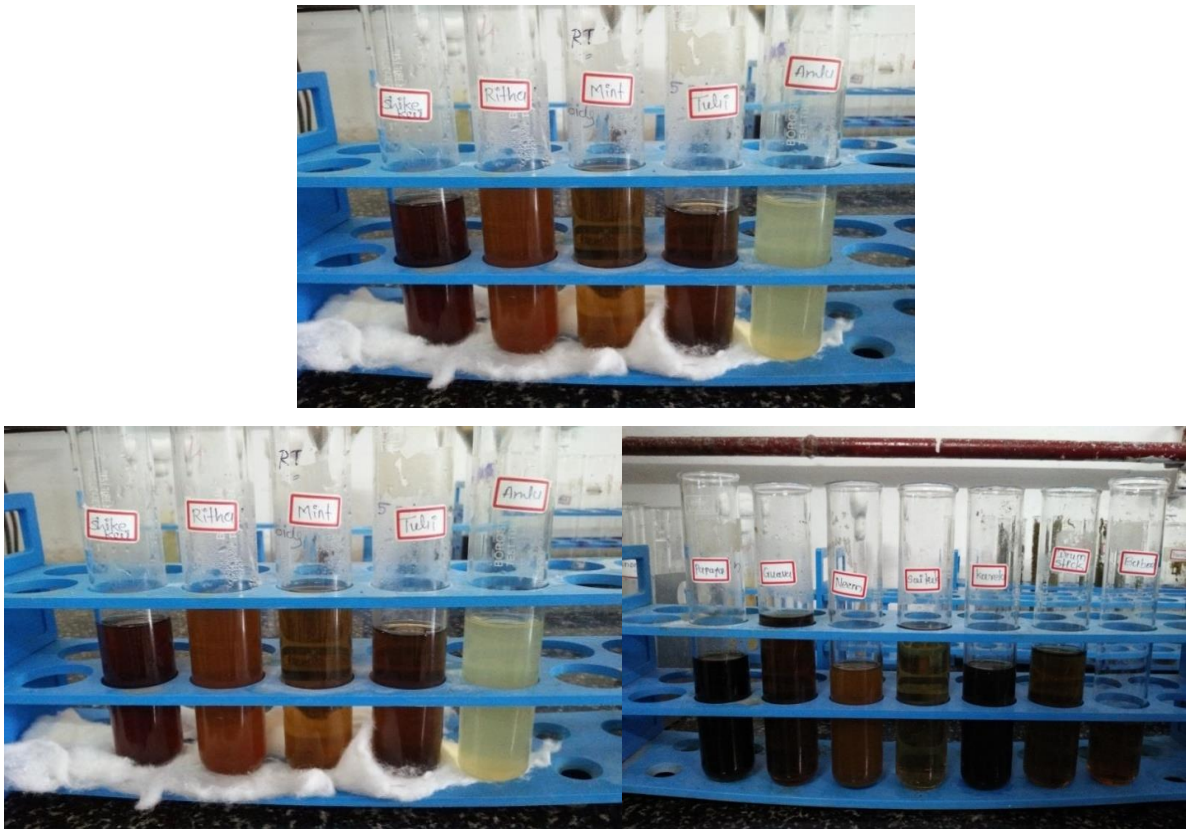

Figure 2: Liquid extracts of all selected medicinal plants.

Table 1: Botanical information of the selected medicinal plant species for phytochemical analysis in Lakhani area of Maharashtra (India).

Sr. no.	PLANT SPECIES	LOCAL NAME	PART USED
1.	*Aloevera barbadensis*	Aloevera	Leaves
2.	*Phyllanthus emlica*	Amla	Fruit
3.	*Osmium santum*	Tulsi	Leaves
4.	*Spndus mukorassi*	Ritha	Fruit
5.	*Mentha spicata*	Padina	Leaves
6.	*Acacia concinna*	Shikekai	Legumes
7.	*Tamarindus indica*	Imli	Fruit
8.	*Curcuma longa*	Haldi	Leaves
9.	*Vichella nilotica*	Babool	Leaves
10.	*Moringa oleifera*	Mungna	Leaves
11.	*Momordica charantia*	Karela	Leaves
12.	*Morus nigra*	Saituk	Leaves
13.	*Azadirachta indica*	Kadunimb	Leaves
14.	*Psidium guajava*	Jamb	Leaves
15.	*Carica papaya*	papai	Leaves

METHOD

1. Test for Coumarins

2 ml of extract treated with 3 ml of 10% NaOH. Formation of Yellow color indicates presence of coumarins.

2. Test for Leucoanthocyanins

Take 5 ml extract treat with 5 ml of isoamylalcohol. Appearance of upper layer Red in color indicates presence of leucoanthocyanins.

3. Test for Phlobatannins

Dry extract shake with water filter it. Then add 1% HCL then boil.

Formation of Red color indicates presence of phlobatannins.

4. Reducing sugar Test

Take 0.5 gm of extract in 5 ml of distilled water. Mix it with 1 ml of Ethanol then add 1 ml Fehling solution A and B in separate test tube and boil. Mix the ethanol extract in liquid test tube. Observe the color reactions.

5. Test for Terpenoids

Take 0.8 gm of sample pour 10 ml of Methanol in it. Shake it well and filter it. Take 5ml of extract mix with 2 ml of Chloroform and add 3 ml of Sulphuric acid. Formation of reddish-brown color indicates positive test.

6. Test for Saponin

2 ml of extract and 6 ml of water added in tube then shake it vigorously. Presence of foam on upper layer indicates positive results.

7. Test for Flavonoids

Take 0.5 gm of extract and 10 ml of distilled water in a tube. Take 5 ml of the extract from it then add 5 ml of Ammonia and 1 ml of concentrated H_2So_4 in it. Appearance of the Yellow color indicates positive results.

8. Test for Quinones

1 ml of extract added to 2 ml of diluted NaOH. Formation of Blue- Green or Red color confirms the presence of Quinones.

PART 2: **For antidandruff activity**

Fifteen different types of plant extracts contains various active compounds which have antifungal activity were used and some chemical based shampoo was used. The inhibitory action was studied using agar well diffusion method and the results indicated by measuring the zone of inhibition.

Method of cultivating organism

Organism collection: The organism was isolated from scalp of persons suffering from Dandruff Flakes or scales, collected by partitioning the hair with a sterile comb and scrapping approximately one inch area using a sterile scalpel. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. There are many techniques for the antifungal tests; the pour-plate test, the spread plate test, disc-diffusion method and well diffusion test. In this study, we used well diffusion method as follows

Culture media preparation: The culture media used was Sabouraud Dextrose Agar (SDA) which was prepared and sterilized in an autoclave at 121°C at 15psi for 15mins.

Identification under microscope: A drop of 10% KOH was added onto a clean slide containing the smear of sample and the smear was covered with a cover slip. The slides were viewed under 40X objective lens.[25]

Culture: The collected samples were cultured on SDA which was incorporated with chloramphenicol to discourage the growth of bacterial contaminants. Small amounts of the samples collected were introduced into petri dishes containing the media using sterile forceps. The petri dishes were labelled and Wrap the edges of petriplates with parafilm, incubated at 30 °C for 7 days.[26]

Staining with Lactophenol cotton Blue

A drop of lactophenol cotton blue was introduced at the centre of a clean slide. fragment of the fungus colony 2-3mm from the colony was collected using inoculating loopand the fragment was droped on the stain and a cover slip was applied and examined under low and high magnifications.

Preparation of plant crude extract: Collected plant material was washed with sterile distilled water and and crushed and finely chopped. Then the extract was filtered, centrifuged and the supernatant was used for antidandruff activity.

Antifungal activity of plant extracts: The antifungal activity fifteen plant extracts on Malassezia spps. was investigated where the agar is poured into a petri dish and allowed to cool then the organism was spread uniformly over the agar surface. After making lawn culture on media plate, make the wells at the center of the media plate with the help of borer. Then transfer the 0.1 ml of the liquid extracts of selected medicinal plants serially in their

respected well. Wrap the edges of petriplates with parafilm/medical tape. Incubate the plates at room tempreture for 2-3 days and then observe the plates for the zone of inhibition.

RESULT

A) Photochemical analysis

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, reducing sugars, flavonoids, phlobatannins were present in the samples. The result of phytochemical analysis shows that the fifteen plants are rich in at least one of the flavonoids, terpenoids, reducing sugars and phlobatannins. Plant *Psidium gujauva* having all these phytochemicals. The phytochemical screening and qualitative estimation of fifteen medicinal plants identified by the recent studies in the local plant Lakhani in which that the leaves were rich in phlobatannins, terpenoids, flavonoids and reducing sugar (Table No. 2).

Phlobatannins are present in *Psidium gujava, Osimum santum* and *Morus nigra.* Phlobatannins have been reported for its wound healing properties, these are anti-infflammatory.[21] and analgesics and antioxidants.[22] Reducing sugars are present only into plants out of fifteen plants i,e, *Psidium guajava, Aloe Vera barbadensis, Phyllanthus emblica, Mentha spicata, Acacia concinna, Vichella nilotica.* Terpenoids were present in *Spindus mukorassi, Acacia concinna, Curcuma longa*, Morus nigra, Azardichta indica, Psidium guajava are reported to have anti-inflammmatory, antiviral, antimalarial, inhibition of cholesterol synthesis and antibacterial.[23] Flavonoids are found in *Aloevera barbadensis, Phyllanthus emblica, Acacia concinna, Momordica charantia, Azadirachta indica, Psidium guajava.* Epidemiologic studies recommended that coronary heart disease is opposed by dietary flavonoids.[19]

Coumarin were present in *Aloe Vera barbadensis, Phyllanthus emblica, Osimum santum, Mentha spicata, Azardichta indica* are reported as chemical defense against predator, used to inhibit formation of blood clot so it is udes in therapy for deep vein thromobosis.(Emeka Kesieme et.al)

Saponin were present in *Aloe Vera barbadensis, Phyllanthus emblica, Spindus mukorassi, Mentha spicata, Acacia concinna, Tamarindus indica, Vichella nilotica, Moringa oleisera, Momordica charantia, Psidium guajava* are reported in health benefits such as cholesterol reducers, also reduce cancer risk, immunity booster and also acts as antioxidant. Saponin

have detergent properties which lead to their use in shampoos,facial cleansers and cosmetic creams.(Mariangela Marrelli et. al).

Quinones were present in *Tamarindus indica, Curcuma longa, Azardichta indica, Morus nigra*. In recent years, quinones has been receiving much attention owing to its physiological importance and pharmacological effects such as growth-promoting activity, anti-diabetic effect, anti-oxidative action, and neuroprotective function.[24]

The recent study of the phytochemical tests of *Moringa oleifera* showed only saponin present in it while other phytochemical components absent in it.[20]

Table 2: Preliminary phytochemical analysis medicinal plant species.

Sr. no./ Medicinal plant	Coumarin	Leucoanthocyanin	Phlobatannin	Reducing sugar	Terpenoid	Saponin	Flavonoid	Quinone
1.Aloevera	+	-	-	+	-	+	+	-
2. Amla	+	-	-	+	-	+	+	-
3. Tulsi	+	-	+	-	-	-	-	-
4. Ritha	-	-	-	+	+	+	-	-
5. Mint	+	-	-	+	-	+	-	-
6.Shikekai	-	-	-	+	+	+	+	-
7.Tamarind	-	-	-	-	-	+	-	+
8.Turmeric	-	-	-	-	+	-	-	+
9. Babool	-	-	-	+	-	+	-	-
10.Drum-stick	-	-	-	-	-	+	-	-
11. Karela	-	-	-	-	-	+	+	-
12. Saituk	-	-	+	-	+	-	-	+
13. Neem	+	-	-	-	+	-	+	+
14. Guava	-	-	+	+	+	+	+	-
15.Papaya	-	-	-	-	-	-	-	-

B) Anti dandruff activity

Out of fifteen different selected plants, Babool showed highest zone followed by Aloe vera, Mint drumstick, Karela and less than this Amla, Shikekai, Turmeric, Tulsi, Tamarind, Neem sbowed minimum zone of inhibition as well as Saituk and papaya showed very less zone of inhibition. In study of chemical based shampoo, head and shoulder and Loreal showed maximum zone of inhibition. In comparision with chemical based shampoo that is Tressemme and Dove, Plant extract Babool,. aloevera, Mint, Drumstick, Karela showed highest zone of inhibition. The inhibition zones of antidandruff shampoos almost matched with those of plant extracts but the sum effect of the later were greater.

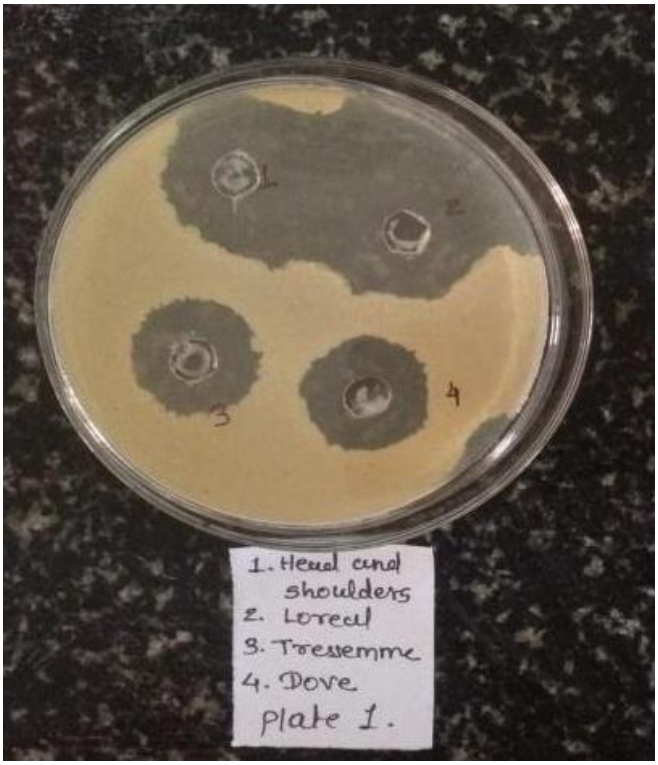

Figure 3: Zone of inhibition of chemical based shampoo.

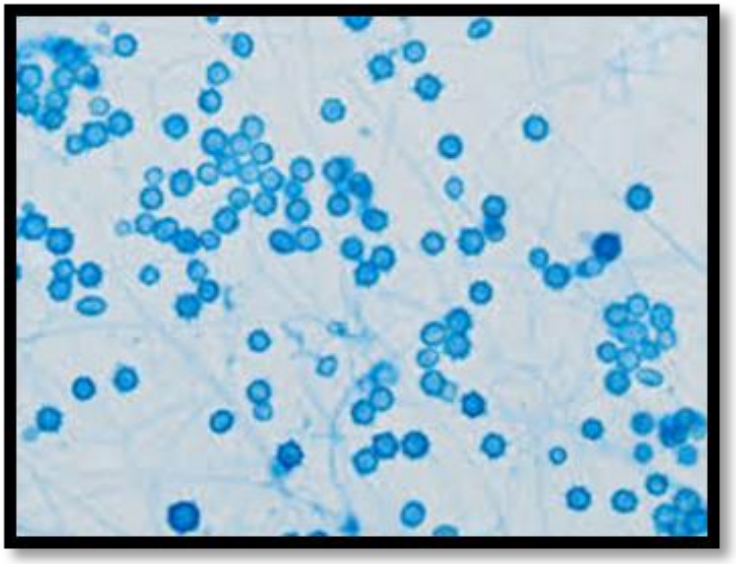

Figure 4: Microscopic view of fungal isolates.

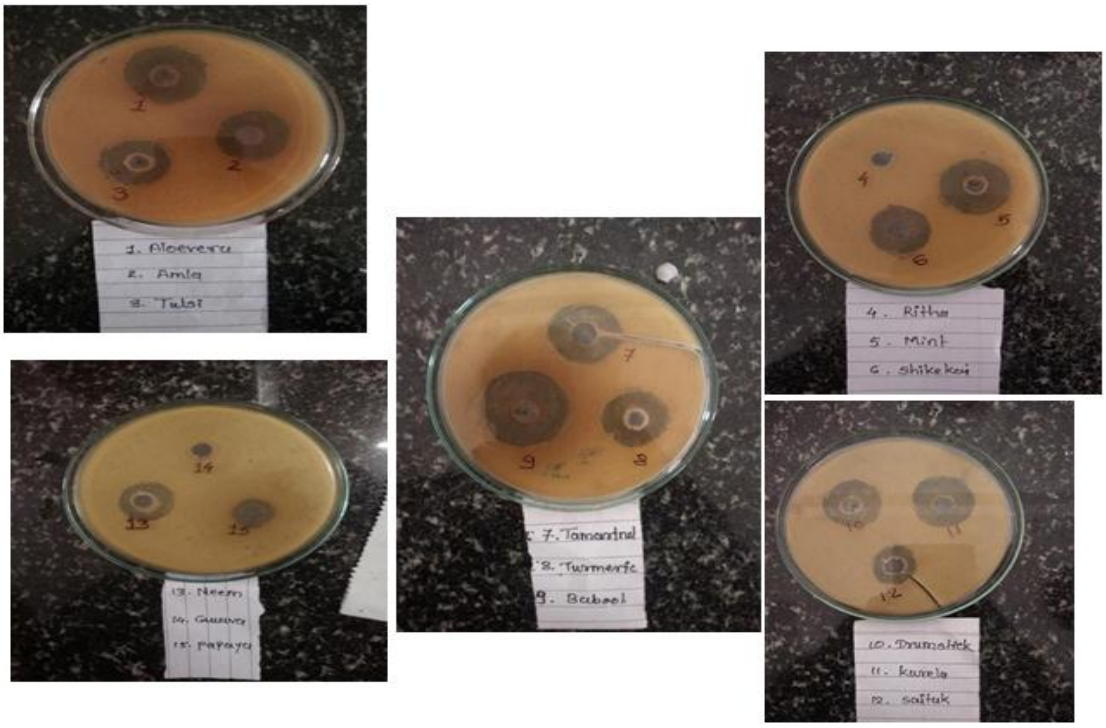

Figure 5: Anti-dandruff activity of all selected fifteen medicinal plants.

Table 3: Plate No. 1:- Chemical shampoos.

Name of the shampoos	Zone of inhibition (mm)
1. Head and Shoulders	30
2. Loreal	34
3. Tressemme	24
4. Dove	25

Table 4: Anti- dandruff activity of selected fifteen plants.

Sr. No.	Name of plants	Zone of inhitibition (mm)
1.	Aloevera (*Aloevera barbadensis)*	26
2.	Amla (*Phyllanthus emblica)*	23
3.	Tulsi *(Osmium santum)*	21
4.	Ritha *(Spindus mukorassi)*	-
5.	Mint *(Mentha spicata)*	25
6.	Shikekai *(Acacia concinna)*	22
7.	Tamarind *(Tamarindus indica)*	21
8.	Turmeric *(Curcuma longa)*	22
9.	Babool (*Vichella nilotica)*	29
10.	Drumstick *(Moringa oleifera)*	24
11.	Karela *(Momordica charantia)*	24
12.	Saituk *(Morus nigra)*	17
13.	Neem *(Azadirachta indica)*	20
14.	Guava *(Psidium guajava)*	-
15.	Papaya *(Corica papaya)*	13

DISCUSSION

A) Photochemical analysis: The research work was carried out on the fifteen selected medicinal plants which shows that phytochemical constituents i.e., coumarins, leucoanthocyanins, phlobatannins, reducing sugars, terpenoids, saponins, flavonoids and quinones are either present or absent in these plants and and the results were summarized in table no.2.

In my study, it was investigated that phlobatannins, terpenoids and flavonoids were found to be absent in extract of *Psidium guajava* whereas in previous studies it was reported that phlobatannins, terpenoids and flavonoids were found to be present in it. The recent research studies and previous research studies results were different so it might be due to the change in location and genetic variation due to cross pollination, so their genetic makeup were changed and that is why shows different results.[3] In the previous work alkaloids were present in *Morus nigra* and the remaining phytochemical constituents were absent. But, recent research work investigation results showed phlobatannins, terpenoids, and quinine in it. From previous research work it was reported that in the leaves of *Morus nigra.*[27] It was reported that the ethanolic / aqueous extract of *Momordica charantia* contained alkaloids, flavonoids and phlobatannins.[28] while in our research investigations flavonoids and saponins were found to be present in it.

B) Anti dandruff activity

Dandruff is a common disease caused by Malassezia species especially Malassezia furfur. The lipolytic activity of these organisms induces hydrolysis of human sebum triglycerides in to free fatty acids that cause both hair loss and scalp. Medically significant fungi media are known to grow on Sabouraud's agar medium. The present isolate being lipolytic grew well on growth of Malassezia species. All the antidandruff shampoos had good antifungal activity but there is considerable variation in the potency of their antifungal activity depending on the active compound (Obasi Chinelo J.et al). The present isolate being lipolytic grew well on growth of Malassezia species. All the antidandruff shampoos had good antifungal activity but there is considerable variation in the potency of their antifungal activity depending on the active compound. This work related to the work of previous author,[29] who reported that the causative organism isolated as Malassezia furfur and further studied the antifungal activity of various plant extracts and synthetic shampoo on the organism and found out that the more zone of inhibition was shown by plant extracts than synthetic shampoo with sun flower

(30mm using water as extract) having the highest zone of inhibition followed by lemon (27mm), aloe vera (17mm), lime (15mm), hibiscus plant and neem plant (10mm) while the best antidandruff shampoo was Vivel Ultra Pro (20mm) as it contains Ketocanozole which is reported to be anti-malassezial agent. This was followed by Dove (14mm) and Head and Shoulders (10mm) as they contain antifungal compounds like Zinc Pyrithione therefore comparatively the plant extracts showed a high zone of inhibition than the shampoos.

The inhibition zones of antidandruff shampoos at low concentrations almost matched with those of plant extracts. On comparison one can say that the plant extracts showed a considerable activity against ruff causing organism Malassezia spps. and can be used to treat dandruff which causes no side effects.

CONCLUSION

A) Photochemical analysis

The selected fifteen medicinal plants are the source of the secondary metabolites i.e Coumarin, Leucoantho Cyanin, Phlobatannin, Reducingsugar, Terpenoid, Saponin, Flavinoid, Quinone. Medicinal plants play a lead role in preventing various diseases. The anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents in Lakhani region Maharashtra, which are very helpful for the manufacturing of new drugs.

B) Anti dandruff activity

Herbal products are cost effective. Prope use of herbs for anti-dandruff condition can reduce the side effects and irritation potential of chemicals to the large extent.

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