IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2018 Vol.:12, Issue:1

Enthralling the Antidandruff Potential of *Ziziphus jujuba* Leaf and Seed Extracts and Formulating a Bioactive Shampoo on *Malassezia* Species

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Santhi. R[1*], Brindha Durairaj[2], Manobharathi. V[3]

[1]Assistant Professor, [2]Associate Professor, Department of Biochemistry,*

[3]Department of Biochemistry, [1,2,3]PSG College of Arts & Science, Coimbatore - 641 014

Submission: 27 March 2018
Accepted: 03 April 2018
Published: 30 April 2018


www.ijppr.humanjournals.com

Keywords: Antidandruff, *Ziziphus jujuba*, *Malassezia furfur*, DNA Fragmentation

ABSTRACT

This study aimed to isolate and identify dandruff causing pathogen and to treat dandruff with *Ziziphus jujuba* leaf and seed extract by *in vitro* methodology by the development of antidandruff shampoo. The seed of *Sapindus trifoliatus* powder was used as biosurfactant for developing shampoo. The formulated shampoo was added to the wells of *Malassezia furfur* inoculated plates. The results were showed that the formulated shampoo has antidandruff activity. DNA fragmentation assay also performed. Thus, it results in lysing *Malassezia furfur*. The damaged DNA witnessed through migration principle-electrophoresis using Agarose as the stationary phase.

INTRODUCTION

Dandruff is a very common skin condition that nearly all people experience at some point in their lives regardless of age or ethnicity. Dandruff typically looks like dry, fine flaky skin on the scalp. Dandruff may improve in summer (as an ultraviolet ray from sunlight counteracts *Pityrosporum ovale*) and may get worse in winter other contributing factors include fatigue, emotional stress, acne, hormonal imbalance, constant exposure to dry air, trauma (scratching), season, use of lotions that contain alcohol and also in some neurological conditions. Several studies on the prevalence of dandruff across the world have shown a prevalence of dandruff up to 50% in the general populations (Ranganathan and Mukhopadhyay, 2010).

Presence of fragments, itching of the scalp and Redness around the scalp is the various symptoms of dandruff (Anusha potluri *et al.*, 2013). Dandruff has several possible causes including dry skin, irritated, oily skin (Seborrheic, dermatitis), not shampooing often enough, other skin conditions like eczema, psoriasis, etc., Mohamed *et al*., (2009) prepared and evaluated an antidandruff herbal shampoo powder using natural ingredients with *Ocimum sanctum* (Tulsi) and *Azadirachta indica* (Neem). They reported that the powder contains all good characters of an ideal shampoo and it was found to be harmless, more effective and economic against strains of gram +ve, gram –ve organism and fungal organism such as *Candida albicans*. Likewise, herbal shampoo using bahera, amla, neem, tulsi, shikakai, henna, and Brahmi were formulated by Sachin *et al.,* (2004) for hair care.

M. furfur and *M. globosa* were found as the most susceptive organism against the aqueous extract of *Phyllanthus Emblica, Hibiscus rosa sinensis, Acacia concinna* and azole drugs (Sibi *et al*, 1996). Globally researchers are using extracts of plants for their antibacterial, antifungal, and antiviral activities (Bakht *et al*, 2011). Tobacco, nicotine inhibits the growth of pathogens which is dose-dependent (Suresh *et al*, 2008).

MATERIALS AND METHODS

Collection of sample

Fresh leaves and seeds of *Ziziphus jujuba* were collected from wayside vendors, Neyveli, Cuddalore district, Tamil Nadu, during the month of December.

Processing of sample

The raw leaves and seed sample were shade dried for 24 hrs, at 40℃ in the hot air oven. After that, they were powdered using a blender.

Methanolic cold extraction

15g of powdered leaves and seed were added with 150 ml methanol separately, stirred in magnetic stirrer for few minutes, and kept for 5 days with intermittent juddering. Then the extracts were kept for evaporation in the water bath at 65℃ to concentrate the extract. Then the extract was weighed and resuspended in methanol.

Phytochemical screening

Constituents of *Z.jujuba* extracts were screened for Alkaloid, Flavonoid, Tannin, Phenol, Terpenoids, Saponin, Protein, Glycoside, Lactone, and Fixed oil.

Isolation of *Malassezia sp.*

Three different samples were collected from different volunteers scalp using the sterile cotton swab and was inoculated on sterile PDA plates by swabbing. The plate was kept at 29℃ for 2 days. After incubation, the colony were observed and is isolated.

Identification of fungi

Lactophenol cotton blue staining

The method followed with the addition of the loop of culture on a drop of lactophenol cotton blue stain exists on the slide. Place a coverslip over it and observe cell morphology under 40x.

Shampoo preparation

Shampoo was prepared according to Ali *et al.* (2011). 2% seed powder of *Sapindus trifoliatus* used as detergent and moisturizer and 1% *Z. jujuba* methanolic extract of leaf and seed each as an antidandruff agent.

Antifungal activity

Well diffusion method

Fungal culture was inoculated in potato dextrose agar and the test solution was loaded into it at 50, 100, 150, 200 µl and is incubated for the overnight at 29℃. It was observed for the zone measurement was made using Hi antibiotic zone scale and the readings were noted in mm.

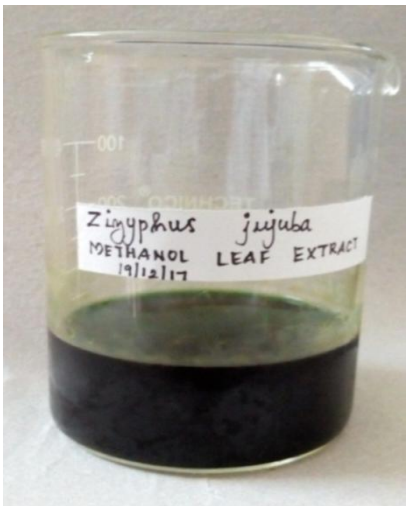
Nucleic acid (DNA) fragmentation assay

The nucleic acid fragmentation assay developed in accordance to determine the efficacy of the *Z. jujuba* methanolic leaf and seed extract to act upon the DNA of dandruff causing yeast as to destabilize its genetic material thus lysing *M. furfur* yeast. The damaged DNA witnessed through migration principle-electrophoresis using Agarose as the stationary phase. The preparation proceeded by applying the herbal shampoo into the overnight culture of *M. furfur* and kept for 1-hour incubation at 37°C and after the incubation period completed the cells was harvested by centrifuge and the total DNA isolated using CTAB.

RESULTS AND DISCUSSION

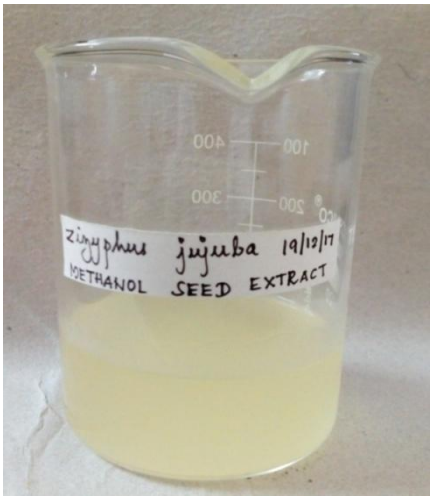
The potent bioactive substance present naturally in the plant leaf and seed (Figure 1, 2 and Table 1) was extracted for those bioactive substances and evaluated for its phytochemical screening and it was found that majority of the phytochemicals were present both in the leaf and seed extract (Figure 3, 4 and Table 2).

Figure 1



Zizyphus jujuba **(Methanol Leaf Extract)**

Figure 2



Zizyphus jujuba **(Methanol Seed Extract)**

Table 1 Quantity of *Z. jujuba* leaf and seed

Source	The quantity of Powder (g)	Initial weight (g)	Final weight (g)	Quantity (15g)	Yield %
Leaf	15	196.7214	199.3617	2.6403	17.60
Seed	15	194.3627	195.1812	0.8195	5.46

Figure 3

***Zizyphus jujuba* Leaf Paper Test**

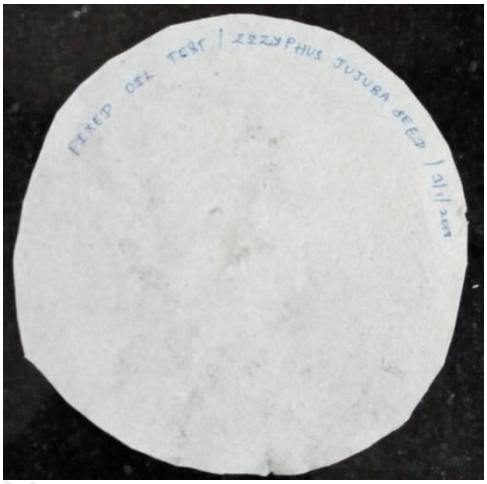


Figure 4

***Zizyphus jujuba* Seed Paper Test**

Table 2 Screening of secondary metabolites of the methanolic extract

Test	Seed Extract	Leaf Extract
Alkaloid	-	-
Phenol	-	-
Tannin	-	+
Saponin	-	+
Flavonoids	+	-
Terpenoids	-	+
Protein	+	-
Glycosides	+	+
Lactone	+	-
Fixed Oil	-	+

The antidandruff activity was evaluated with two preparations as leaf extract and Seed extract as Shampoo. Commercially available dandruff shampoo was used as a positive control. The activity towards the isolated scalp fungi determined by well diffusion method initially with the extracts separately (Plate 1 & 2) and the combined form with surfactant (No zone of inhibition observed in the seed powder of *S. trifoliatus* (Plate 3). The nutrient medium Potato dextrose agar facilitated the growth of *M. furfur*. The extracts are loaded in the well with a concentration range from 12.5mg, 25mg, 37.5mg and 50mg and 5mg, 10mg, 15mg and 20mg respectively. After the incubation period, the fungicidal activity of formulated *Z. jujuba* leaf and seed extracts were observed. The zone of clearance was measured using Zone inhibitory scale (HIMEDIA) and the fungicidal activity was noted in mm (Table 3). MIC was calculated which showed a significant increase in Leaf shampoo were the MIC is 61mg ml and for seed extract shampoo is 64 mg/ml.

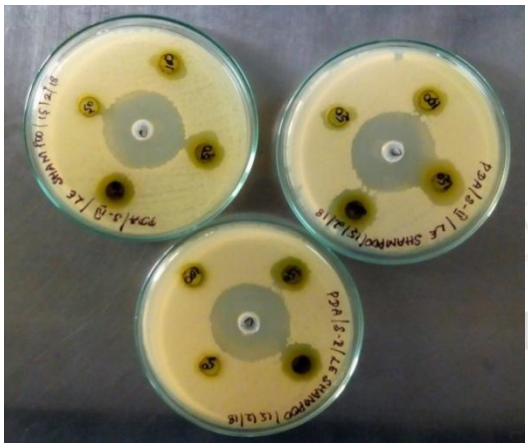


Plate 1 PDA Leaf Extract Shampoo

Plate 2 PDA Seed Extract Shampoo

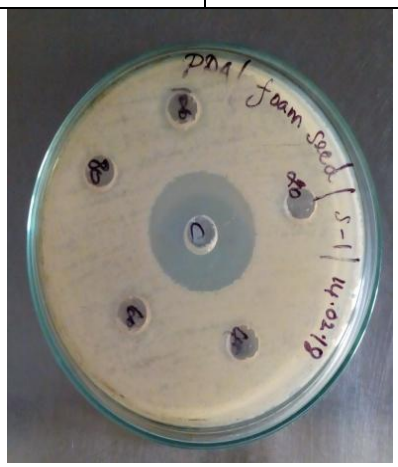


Plate 3 PDA Foam seed

Table 3 Representation chart of MIC - Antidandruff activity

	Volume(µL) with Concentration(µg)					
LE Shampoo	**Control-Dandruff Shampoo**	**50µL**	**100µL**	**150µL**	**200µL**	**MIC (mg/ml)**
		12.5mg	**25mg**	**37.5mg**	**50mg**	
Sample 1	34	10	11	14	17	61.94
Sample 2	33	11	12	17	19	61.51
Sample 3	34	10	12	15	16	61.87
SE Shampoo	**Volume(µL) with Concentration(µg)**					
	Control-Dandruff Shampoo	**50µL**	**100µL**	**150µL**	**200µL**	**MIC (mg/ml)**
		5mg	**10mg**	**15mg**	**20mg**	
Sample 1	35	13	15	15	17	64.42
Sample 2	34	12	13	15	18	64.53
Sample 3	34	12	13	14	20	64.49

The scalp forming yeast was isolated from the affected persons by swabbing and immediately inoculated, the colonies were noted after the incubation period, and the total CFU was also calculated (Table 4). The colonies are pin headed, white and convex and tiny (Plate 4).

Plate 4 Fungal colony of Malassezia species on Potato Dextrose Agar determination of Colony Forming Unit

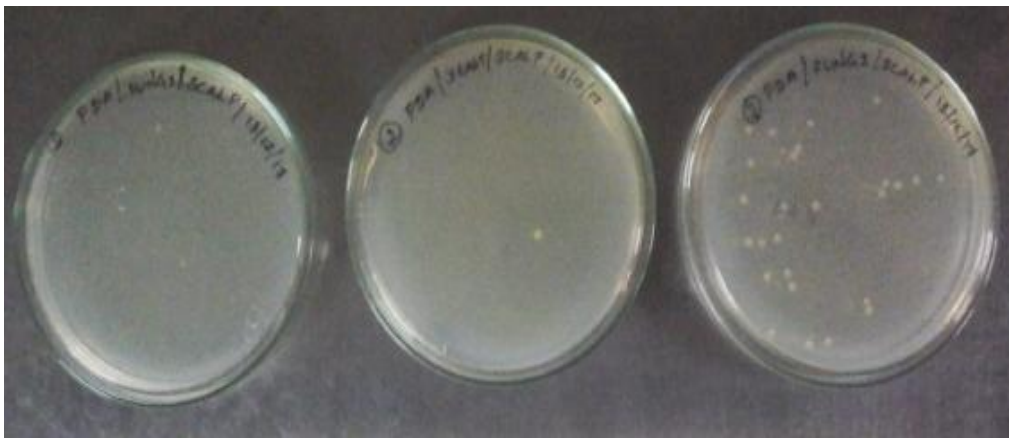


Table 4 CFU detection of *Malassezia species*

Sample Label	Total No. of Colonies	CFU /ml
1	06	3
2	02	1
3	29	14.5

The isolated colonies were microscopically observed to identify the morphological characters of the Dandruff causing yeast using LPCB wet mount at 40 X (Plate 5,6 &7)

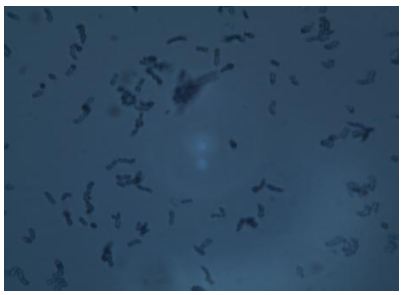


Plate 5 Identification of Yeast – LPCB Staining-Sample 1

Plate 6 Identification of Yeast – LPCB Staining-Sample 2

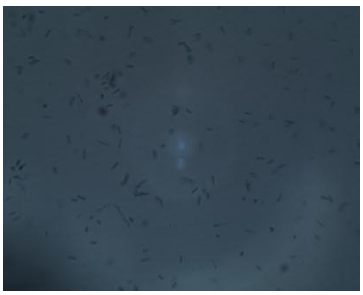


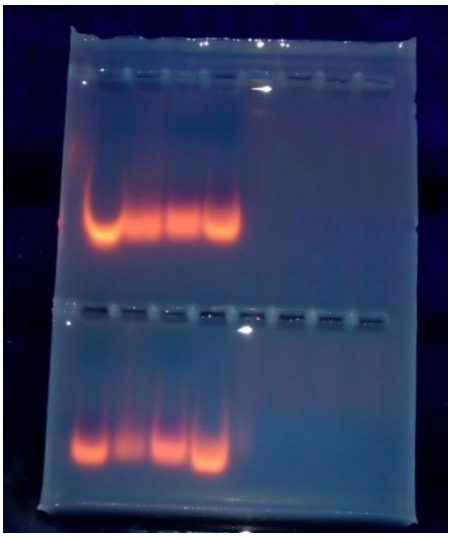
Plate 7 Identification of Yeast – LPCB Staining-Sample 3

The DNA fragmentation assay was performed to elucidate the mechanism of action at nucleic acid level by the fungicide used. To elaborate the formulated surfactant with leaf and seed extracts of *Z. jujuba* was added with log phase culture of *M. furfur* and allowed for incubation. The cell was harvested and isolated for its total genomic content using CTAB and the DNA was separated using 0.8 % Agarose and the band was observed in UV gel documentation system. The band was observed to be damaged by smear formation compared to a positive control DNA that showed a clear double-stranded DNA (Plate 8 and Table 5).

Table 5 Lane description in Gel

Lane	Sample	Inference
LEAF EXTRACT		
1	+ve control	Unique band
2	S 1	DNA damage
3	S 2	DNA damage
4	S 3	DNA damage
SEED EXTRACT		
5	S 1	DNA damage
6	S 2	Complete DNA damage
7	S 3	DNA damage
8	+ve control	Clear band

Plate 8 DNA Fragmentation Assay



CONCLUSION

The major goal of the project study was to find a natural and permanent remedy for scalp i.e. dandruff a threat to hair. *Z. jujuba* a huge remedy for the dandruff sufferer especially from the leaf and seed has proved in this study. Dandruff is a universal scalp disorder affecting almost half of the postpubertal population of any ethnicity and gender. Besides the chemical substance, currently, herbal treatments are available. Herbal cosmetics are widely used when compared to synthetic cosmetics. In hair cosmetics, synthetic cosmetics lead to various side effects such as toxicity to the eye, over drying of hair and deposition of salt on the hair shaft. Hence, this study aids in choosing the most suitable over-the-counter product available

against dandruff in the Indian market and also helps in comparing while assessing the grade of the new product tested for antidandruff activity.

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