

## Evaluation of Anti-Acne and Anti-Dandruff Activity of Seed Protein Extracts

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Received: 11<sup>th</sup> February, 17; Revised 25<sup>th</sup> April, 17, Accepted: 12<sup>th</sup> May, 17; Available Online: 25<sup>th</sup> May, 2017

### ABSTRACT

*Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as pus-forming bacteria triggering an inflammation in acne and *Malassezia* spp, are the causative agent of dandruff in people who have over active sebaceous glands. The present study was conducted to evaluate antimicrobial activities of seed protein extracts from the seeds like flax seeds, soap nuts and pierre against the acne and dandruff causing organisms. The seed proteins were extracted by homogenizing the seeds powder with phosphate buffer followed by ammonium sulphate precipitation and dialysis for purification of protein. These proteins are used to assess the anti-acne and anti-dandruff activity using well diffusion method. MIC of the effective protein extract is determined.

**Keywords:** Antiacne, antidandruff, seed proteins, well diffusion.

### INTRODUCTION

Over the past 2 decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Many types of molecules with antibacterial activity have been isolated from plant source. The Seeds of plants have been reported to produce a number of peptides and proteins with antimicrobial activities, some of them are flax seeds, pierre seeds and soap nut<sup>1</sup>. Flaxseed or linseed (*Linum usitatissimum* L.) cultivated worldwide has attracted people interest for its many benefits in human health. Flaxseed contains lipid, fiber and protein as ingredients of its seed contents. The major product from flaxseed is oil which is rich in omega 3 fatty acids and helpful in preventing cardiovascular diseases and cancer. Since ancient time, the main usage of edible flaxseed has aimed on its oil, fiber and whole intact seed. Accordingly, little attention has been paid on the other benefits of protein components of the seed. There are only a few studies reported on the characteristics and activity of the protein contents of flaxseed<sup>2</sup>.

*Pongamia pinnata* locally known as karanja is a mangrove plant belonging to genus pongamia and family Fabaceae. Traditionally its bark is used in pile; leaves are effective as medicated bath and rheumatic pains; and the seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis. Roots are used for cleaning gums, teeth, and ulcers also effective in gonorrhea. Flowers used for diabetes. In Ayurveda and unani medicine, used as anti-inflammatory, antiplasmodial, anti-noreceptive, anti-hyperglycemic, anti lipoxidative, anti-diarrheal, anti-ulcer, anti-hyper ammonic and antioxidant<sup>3</sup>. *Sapindus emarginatus* is important indigenous plant with lots of traditional importance belongs to family sapindaceae. It is commonly called as Soap nut tree which is found in most

of the hilly regions of India. The members of genus *Sapindus* are well known for their medicinal values. Due to the presence of saponins, they are known for their surfactant & detergent properties. Seeds of *Sapindus emarginatus* contain anti-inflammatory oil which is traditionally used to purify the blood. Soapnuts are also used as effective aid for the treatment of skin problems like eczema, itching and psoriasis<sup>4</sup>.

Generally, microorganisms like *Staphylococcus epidermidis*, *Propionibacterium acnes* are some of the acne causing organism and *Malassezia furfur*, is one of the organism which cause infection in scalp which leads to dandruff. So, these organisms are used in determining the anti-acne and anti-dandruff activity of seed protein extracts.

### MATERIALS AND METHODS

The soapnut, flax and pongamia seeds (pierre seeds) were taken from local market for protein extraction. The organisms for this study were obtained from MTCC Chandigarh. (*Staphylococcus epidermidis* MTCC435, *Propionibacterium acnes* MTCC1951, *Malassezia furfur* MTCC1374)

#### Protein Extraction

#### Soapnut Seed

The outer hard seed coat was removed and 20 g of kernel is homogenized with 200 ml of 50 mM phosphate buffer, pH 7.6 and then made up to 250 ml with the same buffer. The clear supernatant obtained after centrifugation at 2500 rpm, was purified by Ammonium Sulphate precipitation and centrifuged at 10000 rpm for 30 minutes. The precipitate was dissolved in 250 ml of 50 mM phosphate buffer, pH 7.6. The precipitated protein was purified using dialysis method.

Table 1: Effect of different concentration of pongamia and flax seed protein extracts on *Propionibacterium acnes*.

	Concentration ( $\mu$ l)	Zone of inhibition for pongamia (mm)	Zone of inhibition for flax seed (mm)
<i>Propionibacterium acnes</i>	5	06	00
	10	08	03
	20	12	06
	40	21	08

Table 2: Effect of different concentration of pongamia seed protein extracts on *Staphylococcus epidermidis*.

	Concentration in $\mu$ l	Zone of inhibition for pongamia seed (mm)
<i>Staphylococcus epidermidis</i>	5	00
	10	06
	20	09
	40	12

Table 3: Effect of different concentration of soapnut seed protein extracts on *Malassezia furfur*.

	Concentration in $\mu$ l	Zone of inhibition for soapnut (mm)
<i>Malassezia furfur</i>	5	00
	10	00
	20	04
	40	09

Flax Seed: 20 g of powdered seeds was homogenized with 200 ml of 50 mM phosphate buffer, pH7.6 and then made up to 250 ml with the same buffer. The clear supernatant obtained after centrifugation at 2500 rpm was purified by Ammonium Sulphate precipitation and centrifuged at 10000 rpm for 30 minutes. The precipitate was dissolved in 250 ml of 50 mM phosphate buffer, pH 7.6. The precipitated protein was purified using dialysis method<sup>5</sup>.

#### Pierre Seed

The seeds were defatted using a mixture of chloroform: methanol: acetone (2:1:1) for extraction of soluble protein. Defatted seeds were ground in 50ml 0.12M Tris HCl buffer of pH 6.8 and incubated over night at 4°C. Sample was centrifuged at 15000 rpm for 15mins at 4°C, and pellet was collected and the steps were repeated for complete extraction. Ammonium sulphate precipitation was done and again centrifuged at 10,000 rpm to collect the pellet. The precipitate dissolved in 250 ml of 0.12M Tris HCl buffer. Purification of extracted protein was done by dialysis method<sup>6</sup>.

Quantitative estimation of protein was carried out by Lowry's method.

#### Antimicrobial Test

The bacteria were grown on Nutrient agar, blood agar and yeast in Sabouraud dextrose agar respectively. Inoculum of test bacterial species was prepared by growing pure isolate in nutrient broth and yeast in Sabouraud dextrose broth for 7days. The agar plates were prepared by swabbing the inoculum of respective test organism. The 6mm diameter wells were made using cork borer and 20 $\mu$ l of protein extracts were added. After incubation the diameter of inhibition was measured in mm<sup>6</sup>.

#### Minimum Inhibitory Concentration Test

The proteins which showed inhibition in antimicrobial test were taken to test their minimum inhibitory concentration. Wells of 6mm size were made and 5 $\mu$ l, 10 $\mu$ l, 20 $\mu$ l, and 40 $\mu$ l of extracts were added. The nutrient agar plates were incubated for 24h while Sabouraud dextrose agar plates for 1 week. The diameters of zones of inhibition were measured in mm<sup>7</sup>.

## RESULT AND DISCUSSION

The flax seeds, pierre seeds (pongemia seeds) and soapnut were collected from local market of Davangere. The protein was extracted and purified by salt precipitation and these samples were checked quantitatively by Lowry's method, and concentration of protein was recorded (Flax seeds - 460  $\mu$ g, soapnut - 520  $\mu$ g, Pierre seeds - 470  $\mu$ g). The antimicrobial activity of seeds protein extracts of pierre, soapnut and flax seed was determined. The extract of pongamia seed showed inhibition of 8mm and 10mm for the growth of acne causing organisms like *Staphylococcus epidermidis* and *Propionibacterium acnes* respectively. The extract of flax seed showed inhibition of 9mm for the growth of acne causing organism *Propionibacterium acnes* and the extract of soapnut showed inhibition of 5mm against dandruff causing organism *Malassezia furfur*.

MIC for the effective extracts is shown in table 1,2 and 3. Pongamia seed protein extract were found more effective for *Staphylococcus epidermidis* and *Propionibacterium acnes* with maximum zone of inhibition of 12mm and 21 mm respectively. And this showed seed protein extracts are effective significantly against acne causing organisms. Protein extracted from soapnut seeds was found effective against dandruff causing organism. Acne and dandruff are the major dermatologic problems in youth nowadays caused by some microbes and the proteinaceous material extracted from these seeds have shown anti-microbial properties like anti-acne and anti-dandruff activities.

## CONCLUSION

The seeds like soapnut, flax seeds and pierre seeds are having beneficial effects on human health. The proteins from these showed anti-acne and anti-dandruff property against causal organisms. The further study can be carried out on these proteins for separation and isolation of individual protein and can be used in the formulation of shampoos and acne creams.

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