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Comparative Study of *Averrhoa Bilimbi, Ricinus Communis and Saraca Asoca* Leaf Extracts on Dandruff Causing Fungus and Bacterial Strains

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ABSTRACT

Dandruff is a major condition faced by most of the individuals which is characterised by the itchy scalp with white flakes. It is believed that this happens due to many environmental effects. The main organism involved in the cause of dandruff is a fungal species called *Malassezia*. In this study the phytochemical, antifungal and antibacterial activity of different leaf extracts were analysed. The efficacy of the leaf extracts from *Averrhoa Bilimbi, Ricinus Communis and Saraca Asoca* were tested on dandruff causing fungus and bacterial strain of *E.coli* and *Bacillus* for the study. This comparative study was mainly aimed at using the leaf extracts to check if they could inhibit the growth of fungus and bacterial strains. The antifungal and antibacterial activity wereactivities were carried out using the zone of inhibition method with different concentrations of leaf extracts.

KEYWORDS: Dandruff, *Averrhoa Bilimbi, Ricinus Communis, Saraca Asoca,* Leaf Extracts

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1. INTRODUCTION

Dandruff is a chronic skin condition, especially in the scalp which is mainly characterized by flakes and mild itching. Dandruff is a result of major factors like *Malassezia* fungi, sebaceous secretions, genetic and environmental factors. Usually dandruff seems like small pieces of dead skin in the person's hair. The cause is unclear but believed to involve various genetic and environmental factors. In general, dandruff occurs after puberty and mainly affects males than the females. *Malassezia* converts the sebum lipid into fatty acids and triglycerides, which accelerate hyperproliferation of keratinocytes. The treatment options currently available for management of dandruff have zinc pyrithione, salicylic acid, imidazole derivatives, selenium sulphide, tar derivatives, ketoconazole etc. as key ingredients. These synthetic treatment options have certain limitations, which may be due to poor efficacies or due to compliance issues. These are unable to prevent recurrence of dandruff with side effects that cannot be neglected. The best approach to treat dandruff is to use plants and herbal formulations which possess anti dandruff properties (*Arora et al., 2011*). This study involves the analysis of antifungal activity of leaf extracts of *Averrhoa Bilimbi, Ricinus Communis and Saraca Asoca* against *Malassezia* and antibacterial activity was also analyzed (*Kashyapa K and Chand R, 2006*).

Malassezia is a genus of fungi. It is commonly found on the surface of the skin of many animals, including humans. *Malassezia* is involved in disorders like dandruff, and seborrheic dermatitis, which affects more than 50% of the human population. *Escherichia coli* is a bacterium commonly found in the gut of warm-blooded organisms. Most strains of *E.coli* are not harmful but are part of the healthful bacterial flora in the human gut. However, some types can cause illness in humans, including diarrhea, abdominal pain, fever, and sometimes vomiting. *Bacillus* is a genus of gram positive, rod shaped bacteria, a member of phylum Firmicutes, with 266 named species. Some types of *Bacillus* bacteria are harmful to humans, plants, or other organisms. Most of them are not pathogenic for humans but may infect humans accidentally (*Chhavvi et al., 2011*).

Averrhoa bilimbi (Bilimbi) belongs to the family Oxalidaceae, native to Indonesia, Philippines, Srilanka, Bangladesh, Maldives, Myanmar, Malaysia, and coastal regions of India. The plant parts are used to treat venereal diseases and have anti-inflammatory

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properties. It has antihyperlipidemic properties which controls obesity (*Abraham, 2016*). *Ricinus communis* (Castor) is a perennial flower plant belonging to the family Spurges, grows in the Southern Mediterranean Basin, Eastern Africa and India. It is rich in triglycerides, mainly ricinolein. The plant parts have been used to treat liver disorders, hypoglycemic conditions and exhibit anti-inflammatory, antinociceptive, antioxidant and cytotoxic activities (*Obumselu et al., 2011*). *Saraca asoca* (Ashoka) is a tree of cultural traditions, belonging to the family Fabaceae which grows in the Indian subcontinent (*Lall et al., 2013; Divya et al., 2017*). It is an important indigenous plant with several traditional importance, the parts of Ashoka plant are used for menorrhagia, astringent, diabetes, biliousness, dyspepsia, ulcers, uterine stimulant, estrogenic effect and abortifacient (*Cowan, 1999, Das et al., 2010*).

2. MATERIALS AND METHODS

2.1. Plant material collection and extraction

Leaves of *Bilimbi*, *Castor*, and *Ashoka* were shade dried for 15 to 20 days and then they were ground to powder and sieved using a mesh. About 10g of the powder was extracted by mixing in 100ml of distilled water followed by boiling for 15 minutes. The extract was filtered through Whatman no.1 filter paper to get a clear mixture. This extract was used to perform phytochemical analysis (*Divya et al., 2017*).

2.2. Isolation of the samples

Fungi: Samples were collected from different patients by scraping the lesions and they were streaked using a sterile cotton bud in Potato Dextrose Agar medium (PDA). The petri plates were incubated at room temperature (3 to 4 days) and the growth was recorded (*Vaishali and Nayan, 2018*).

Bacteria: Samples for bacteria (*E. coli* and *Bacillus*) were grown from the mother culture in nutrient agar. Serial dilution was done and the 10^{-2} dilution was taken for culturing using spread plate technique with a sterile L -rod. The plate was incubated for 24 hours in the incubator for the growth of bacteria (*Lupindu, A. M., 2017*).

2.3. Phytochemical Analysis

The Phytochemical analysis was performed as follows (*Nidhi et al., 2013*),

Test for alkaloids: 2ml of 1%HCl was mixed with 1.5ml of extract and heated slightly. After cooling Wayner's reagent was added to it. The formation of buff -colored precipitate indicated the presence of alkaloids.

Test for carbohydrates: 1ml of Benedict's reagents was mixed with 1.5ml of extract and slightly boiled, appearance of reddish-brown precipitate indicated the presence of carbohydrates.

Test for flavonoids: The appearance of pink scarlet color and formation of pellets when 1.5ml of extract was mixed with few drops of conc HCl indicated the presence of flavonoids.

Test for phenols: 2ml of 2% $FeCl_3$ was mixed with 1.5ml of extract and formation of blue-green or black color indicated the presence of phenols.

Test for saponins: Presence of saponins was detected by frothing test. About 5ml of extract was mixed in 2ml of distilled water and kept in a boiling water bath for 5mins and shaken vigorously. The appearance of foam indicated the presence of saponins.

Test for steroids: 1.5ml of extract was mixed with 2ml of H_2SO_4 followed by slow addition of 2ml of acetic anhydride. The color change from violet to green/blue indicated the presence of steroids.

Test for tannins: 1.5ml of extract was mixed in few drops of $FeCl_3$. The appearance of green or blue-green precipitate indicates the presence of tannins.

Test for terpenoids: Equal volume of extract and chloroform was taken. Formation of a reddish-brown layer at the junction of the two solutions confirms the presence of terpenoids.

Test for cardiac glycosides: 5ml of extract was treated/ reacted with 2ml of glacial acetic acid followed by the addition of 1 drop of $FeCl_3$ solution and 1ml of concentrated sulphuric acid. Formation of the brown ring at the interface confirms the test for cardiac glycosides.

Test for proteins: 2ml of extract was boiled with 2ml of 2% acetone solution. Appearance of violet color indicates the presence of proteins.

2.4. Analysis of antifungal activity

Three petri plates were prepared with Potato Dextrose Agar after which the plates were inoculated with culture using a cotton swab by streak plate method. Four wells were punched with one as blank (distilled water) and the other three for the leaf extracts. 10µL of sample (each leaf extract was loaded in one petri plate). Later, the plates were incubated for 24hours and was observed for zone inhibition. The experiment was performed in triplicates (*Pingili M et al., 2016*).

Table 1: Preliminary phytochemical analysis of the plant extracts

Sl. No	Test	Ashoka	Bilimbi	Castor
1	Alkaloids	Positive	Negative	Negative
2	Carbohydrates	Negative	Positive	Negative
3	Flavonoids	Positive	Negative	Positive
4	Phenols	Positive	Positive	Positive
5	Saponins	Positive	Positive	Positive
6	Steroids	Positive	Positive	Positive
7	Tannins	Positive	Positive	Positive
8	Terpenoids	Negative	Negative	Negative
9	Cardiac glycosides	Negative	Positive	Negative
10	Proteins	Negative	Negative	Negative

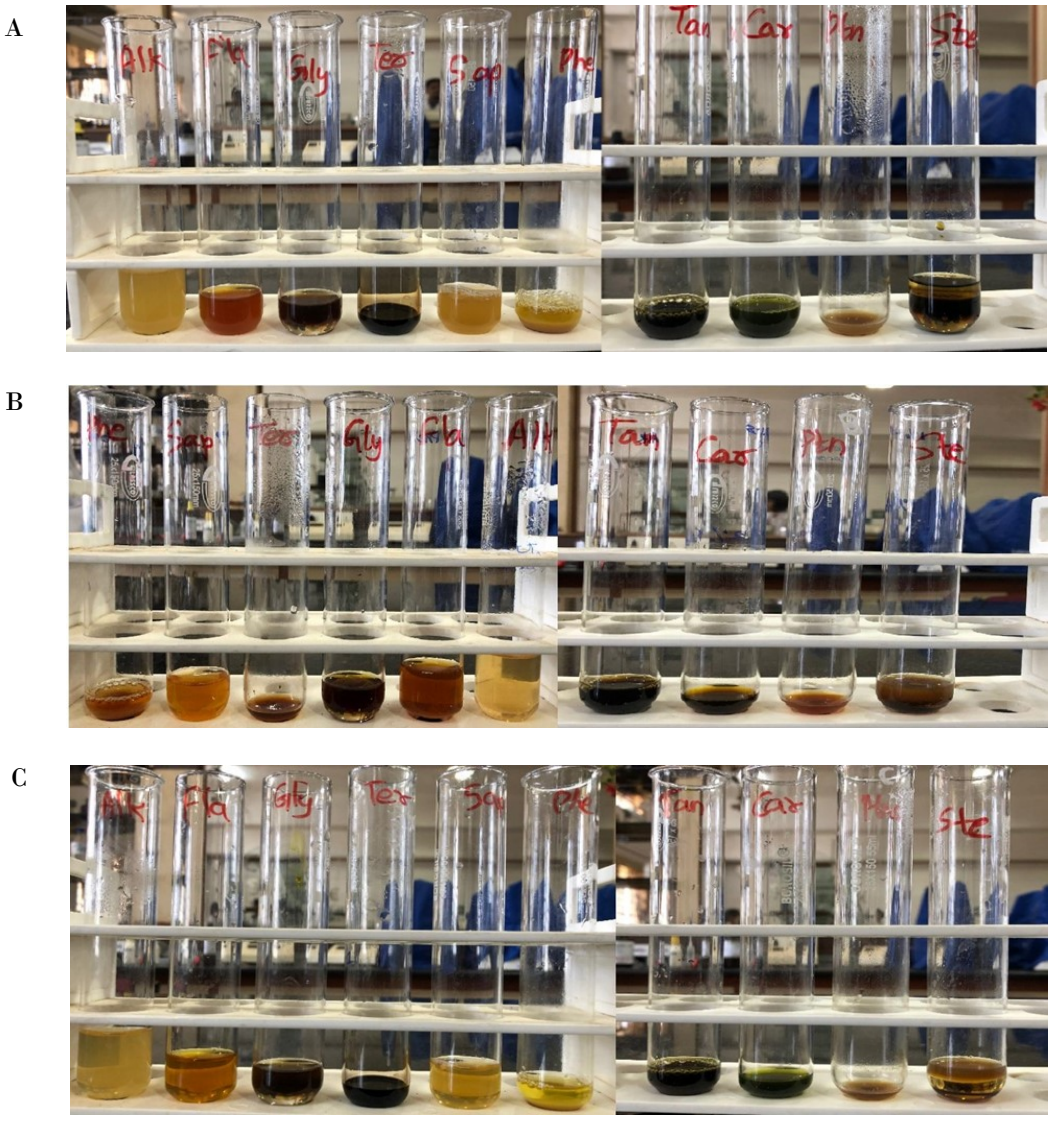

Figure 1: Phytochemical analysis of plant extracts. (A) Ashoka (B) Bilimbi (C) Castor

2.5. Analysis of antibacterial activity

Three petri plates were prepared with nutrient agar, then the plate was inoculated with the bacterial cultures using a cotton swab by streak plate method. Four wells were punched with one as blank (distilled water) and the other three for the leaf extracts. 10µL of sample (each leaf extract was loaded in one petri plate). Later, the plates were incubated for 24 hours and was observed for zone inhibition. The experiment was performed in triplicates (*Sarojini N et al., 2011*).

3. RESULTS

3.1. Phytochemical Analysis: The phytochemical test was conducted using the leaf extracts and the Table 1 shows the results with the presence of phenols, saponins, steroids and tannins in all the three extracts, depicted in Figure 1A. Ashoka, Figure 1B. Bilimbi and Figure 1C. Castor leaf extracts.

3.2. Antifungal Activity: The antifungal test was conducted using the streak plate technique and well punching method. The zone of inhibition method shows that there is no antifungal activity of leaf extract on the fungus, since there was no zone of inhibition as demonstrated in Figure 2. Hence, there was growth of fungal strain on the plates in which the leaf extracts were tested. The zone of inhibition was not seen. Hence can be concluded as negative result for the antifungal activity.

Figure 2: Zone of Inhibition for Fungal strain

3.3 Antibacterial Activity: The antibacterial activity was analyzed by performing the zone of inhibition using the streak plate and well punching method. Development of clear zones were observed for the leaf extracts except for Castor as demonstrated in Fig 3. The zone of inhibition was clearly seen with the diameter of 1cm, 0.5cm and 0cm (Ashoka, Bilimbi, and Castor respectively) for *E.coli* and 1.5cm, 0.7cm and 0cm (Ashoka, Bilimbi, and Castor respectively) for *Bacillus* and hence the extracts were found to have antibacterial activity on a comparative basis while the extract from Castor did not exhibit antibacterial activity. Figure 3 and Table 2 shows the antibacterial activity of the leaf extracts.

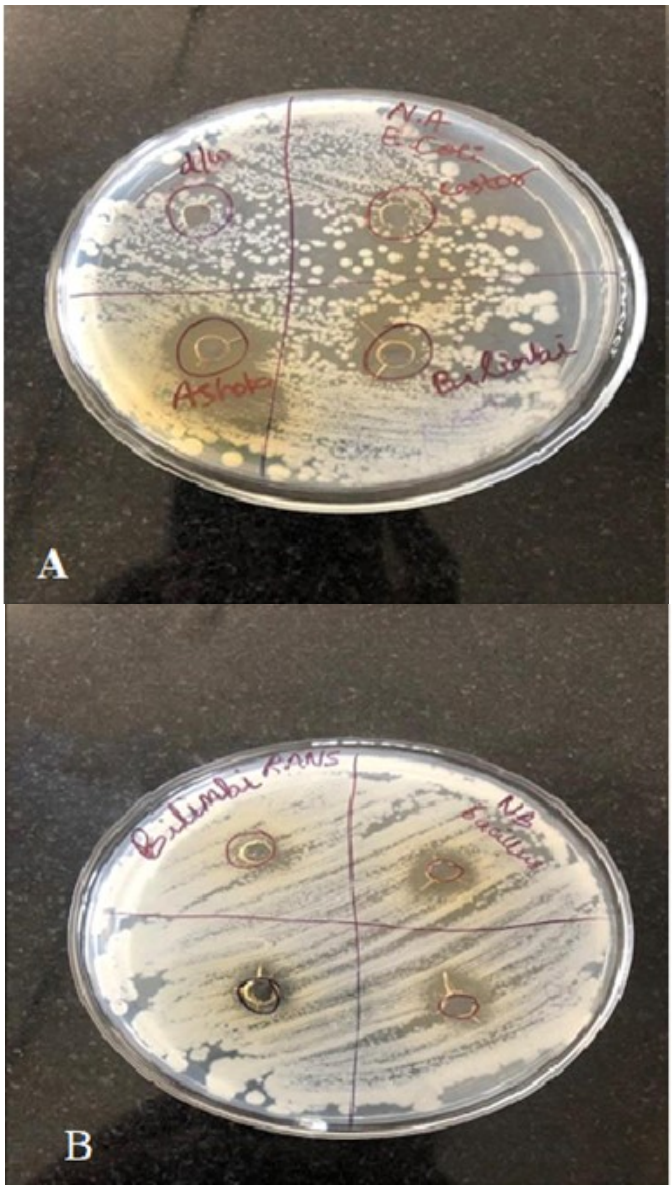

Figure 3. (A) Antibacterial activity of leaf extracts against *E.coli* (B) Antibacterial activity of leaf extracts against *Bacillus*

Table 2: Zone of Inhibition for antibacterial activity of 10 µg leaf extracts

Extract	Zone of Inhibition (cm)	
	E. coli	***Bacillus***
Ashoka	1.0	0.8
Bilimbi	0.5	0.5
Castor	0.0	0.0

4. CONCLUSION

Present study confirms the antibacterial activity of leaf extracts from Ashoka and Bilimbi towards *E.coli* and *Bacillus* strains. Leaf extract from Castor has no effect on the bacterial strains since there was no development of zone of inhibition. From the above results we conclude that leaf extracts did not show any antifungal activity on the dandruff causing fungus. The study also shows the presence of various secondary metabolites in the plants (Ashoka, Castor and Bilimbi). The leaf extracts especially Bilimbi and Ashoka showed good antibacterial activity and hence they can be used for prevention of growth of microorganisms.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest .

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