

## In vitro Antifungal Effects of Hesperetin and Silibinin

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### ABSTRACT

**Background:** Increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. To overcome the resistance and other drawbacks of the current antimicrobial drugs and to obtain more efficacious drugs, an antimicrobial drug having a novel mode of action should be developed. The flavonoid compounds have demonstrated for their therapeutic potential as antimicrobial agents previously.

**Aim:** To evaluate the antifungal efficacy of plant derived compounds such as hesperetin (HSP) and silibinin (SBN) compounds *in vitro*. **Materials and Methods:** In this study, we evaluated the antifungal effect of two plant derived flavonoid compounds *i.e.*, SBN and HSP against *Candida albicans*, *Candida tropicalis*, *Malassezia furfur*, *Aspergillus niger*, *Penicillium oxalicum*. The disk diffusion test was performed to test the antifungal effect of HSP and SBN with three different concentrations *i.e.*, 15, 20, 25 mg/ml of HSP and SBN. The antifungal efficacy was studied for 72 h. **Results:** Our results indicate that SBN has significant antifungal effect than that of HSP. The high sensitivity of these compounds was found against *C. albicans*, *C. tropicalis*. **Conclusion:** These drugs could be good candidate to combine with regular antimicrobial agents.

**Key words:** Flavonoids, Silibinin, Hesperetin, *Candida albicans*, Fungal infection.

## INTRODUCTION

A new spectrum of human fungal infections is increasing due to increased cancer, AIDS, and immunocompromised patients. The increased use of antifungal agents also resulted in the development of resistance and it makes necessary to discover new classes of antifungal compounds to cure fungal infections.<sup>1</sup> *Candida albicans* is a polymorphic fungus which is the predominant cause of invasive candidiasis, superficial and deep tissue fungal infections. In particular, the invasive candidiasis is the most common invasive fungal disease causing an unacceptably high mortality. Some species of *Candida* have emerged as an important cause of severe candidaemia and can exhibit reduced susceptibility to the current antifungal agents.<sup>2</sup> Meanwhile, candidiasis due to *Candida tropicalis* has increased dramatically on a global scale thus proclaiming this organism to be emerging pathogenic yeast.

*Candida tropicalis* is one of the more common *Candida* causing human diseases in tropical countries and it has been identified as the most prevalent pathogenic yeast species of the *Candida*-non-*albicans* group.<sup>3</sup> Resistance to fluconazole in clinical isolates of *C. tropicalis* has increased.<sup>4,5</sup> There are several reports on azole resistance is reported, specifically in *C. albicans* and *C. tropicalis*.<sup>6,7</sup> The *Malassezia* comprises lipophilic species, the natural habitat is the skin of humans and other warm-blooded animals.<sup>8</sup> *Malassezia* species can be involved in skin disorders, such as pityriasis versicolor, seborrheic dermatitis, atopic eczema, and folliculitis, and occur at higher population densities on scalps with dandruff

than on scalps without dandruff.<sup>9</sup> *Penicillium oxalicum*, a plant pathogen and it has been recently found that causes an opportunistic fungal infection in patients with acute myeloid leukemia, diabetes mellitus, and chronic obstructive pulmonary disease.<sup>10</sup>

Increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. It is reported that pathogenic viruses, bacteria, fungi, and protozoa are more and more difficult to treat with the existing drugs due to development of resistant.<sup>11</sup> To overcome the drawbacks of the current antimicrobial drugs and to obtain more efficacious drugs, an antimicrobial drug having a novel mode of action should be developed. Plant-derived flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, tea, and wine. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases.<sup>12</sup> Hence, in this study, we tested the antifungal effect of hesperetin and silibinin against different fungal species.

Silibinin (SBN) is a flavonolignan extracted from the seeds of a medicinal plant *Silybum marianum* (Asteraceae), commonly called "milk thistle".<sup>13</sup> Hesperetin (HSP) is isolated from citrus fruits that have previously been found to exert anticancer activity through a variety of mechanisms.<sup>14</sup> Both HSP and SBN (Figure 1) have been reported to have wide spectrum of biological activities. These plant derived

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flavonoid compounds have been tested for their anti-proliferative, anti-microbial, anti-inflammatory, antioxidant, anticancer, free radical scavenging, membrane stabilizing properties and came out with promising results.<sup>14-17</sup> However, studies regarding their antifungal effect are scanty. Hence, in the present study we evaluated the antifungal efficacy of hesperetin and silibinin against different fungal species.

## MATERIALS AND METHODS

### Reagents and Drugs

Hesperetin (C<sub>16</sub>H<sub>14</sub>O<sub>6</sub>; CAS No. 69097-99-0) was purchased from Sigma Chemicals, India.

Silibinin (C<sub>25</sub>H<sub>22</sub>O<sub>10</sub>; CAS No. 22888-70-6) was purchased from Sigma Chemicals, India. It is a mixture of two diastereomers (silybin A and silybin B). All other chemical used in this study purchased locally and were of analytical grade.

### Fungal species used

*Candida albicans*, *Candida tropicalis*, *Malassezia furfur*, *Aspergillus niger*, *Penicillium oxalicum*

### Experimental design

The fungal cultures in Sabouraud's Dextrose Agar plate were filled with three different concentrations i.e., 15, 20, 25 mg/ml of HSP and SBN. The antifungal efficacy was studied for 72 h.

### Disk diffusion method

The disk diffusion test was performed to test the antifungal effect of HSP and SBN.<sup>18</sup> On sterile plates containing Sabouraud's Dextrose Agar, the fungal cultures were swabbed. Then, sterile discs (made from Whatman filter paper) each about 5 mm diameter impregnated with the HSP and SBN separately was placed on the inoculated plates. Similarly, each plate was placed with a sterile disc. The plates were incubated at 28°C for 72 h for evaluation. The diameter of inhibition zones formed around the disk was measured in millimeter. The test was carried out in triplicate in each run of the experiments.

## RESULTS

### Antifungal activity of HSP

The results from this study clearly show that HSP has antifungal efficacy only against *C. albicans*, *C. tropicalis*, *Malassezia furfur* species. Hesperetin has produced dose-dependent inhibition of *C. tropicalis* and *Malassezia furfur* fungal growth, whereas, in low concentrations hesperetin did not show any effect in the growth of *C. albicans*. The maximum inhibitory effect was seen at maximum dose tested in this study i.e., 25 mg/ml. This flavonoid exhibits higher efficacy against *C. tropicalis* and *Malassezia furfur* when compared to *C. albicans* (Figure 2). Hesperetin did show any significant change in the growth of *Aspergillum niger* and *Penicillium oxalicum*.

### Antifungal activity of SBN

We also tested the antifungal efficacy of the SBN against the *C. albicans*, *C. tropicalis*, *Malassezia furfur*, *Aspergillum niger* and *Penicillium oxalicum*. The results obtained from present study shows that SBN has antifungal efficacy only against *C. albicans*, *C. tropicalis*, *Malassezia furfur* among fungal organism tested. Silibinin shows high sensitivity against *Candida* species i.e., *C. albicans*, *C. tropicalis* when compared to other species (Figure 3). In fact, SBN did show any significant change in the growth of *Aspergillum niger* and *Penicillium oxalicum*. The maximum effect was observed with higher concentration of SBN used in this study.

## DISCUSSION

Human fungal pathogens cause invasive infections are hidden killers, taking lives of 1.5 million people every year.<sup>19</sup> Therapeutic applications of immunosuppressive drugs, the use of broad spectrum antibiotics against various clinical settings are responsible for an increasing number of immunocompromised patients and consequent opportunistic fungal infections worldwide. An impaired immune system provides favorable conditions for pathogenic and non-pathogenic micro-organisms.<sup>3</sup>

Hesperetin, a flavanone glycoside predominantly found in citrus fruits, exhibits a wide array of biological properties.<sup>20</sup> Studies have shown the antimicrobial effect of HSP especially against bacterial strains and came out with promising results.<sup>21</sup> In the present study, HSP shows antifungal activity against *Candida* species i.e., *C. albicans* (only in higher concentration) and *C. tropicalis*. The HSP treatment also shows inhibitory effect against *Malassezia furfur*. On the other hand antimicrobial effect of SBN has also been concretely proved against wide variety of bacterial



Figure 1: Chemical structure of hesperetin and silibinin

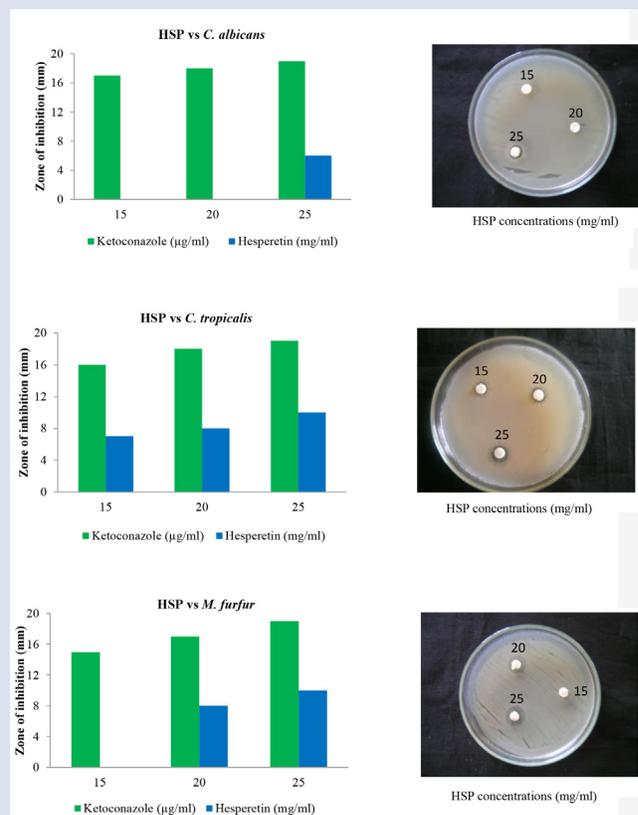


Figure 2: Antifungal effect of hesperetin.

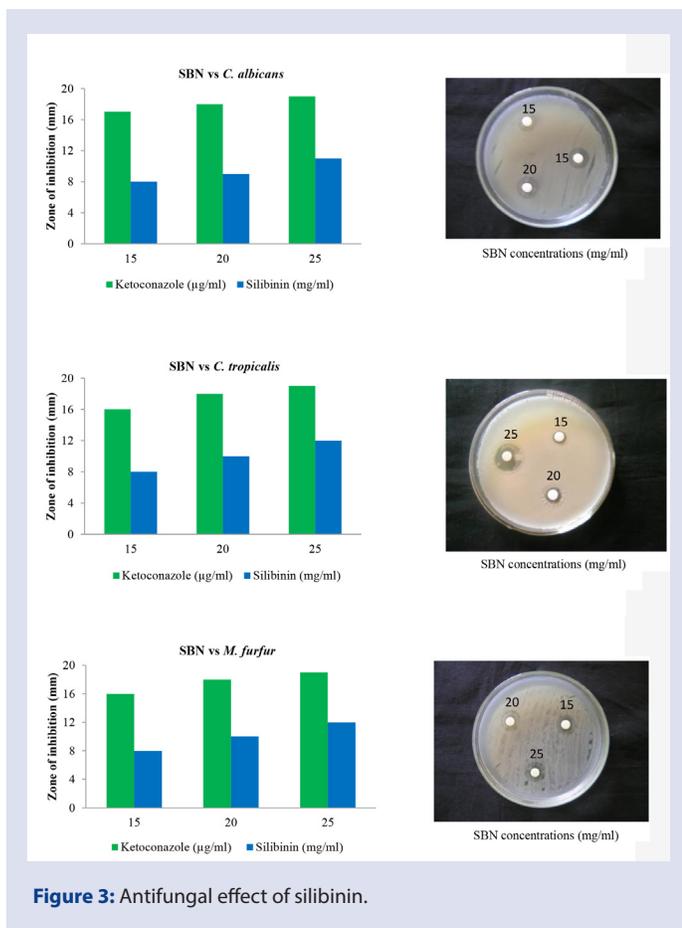


Figure 3: Antifungal effect of silibinin.

strains.<sup>17,22</sup> The antifungal effect of SBN found in this study is marginally greater than HSP. Moreover, SBN shows higher efficacy against *C. albicans* and *C. tropicalis*.

Flavonoids are hydroxylated phenolic substances synthesized by plants in response to microbial infection. Flavones are phenolic structures containing one carbonyl group and the addition of a 3-hydroxyl group yields a flavonol. Flavonoids have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably suggested due to their ability to complex with extracellular and soluble proteins and to complex with fungal cell wall. It also been said that high lipophilic nature of flavonoids also disrupts fungal membranes.<sup>23</sup> In light of the above reports it is suggested that the profound antifungal effect of HSP and SBN found in this study could be due to their action on fungal membrane disruption and subsequent inhibition of its growth.

Collectively, this study clearly shows that SBN possess significant antifungal efficacy than HSP. It should be brought to light that both these flavonoids show efficacy only against *C. albicans*, *C. tropicalis* and *Malassezia furfur* and did not show any efficacy against the *Aspergillum niger* and *Penicillium oxalicum*. Further, it has to emphasize that in previous studies flavonoid compounds have demonstrated for their therapeutic potential as antimicrobial agents. The synergistic effect of flavonoids combined with commonly utilized antibiotics is well supported in the literature and this strategy is emerging as an important complementary treatment modality in research.<sup>17</sup> Accordingly, the present study would be of immensely useful to identify some of the efficacious plant derived flavonoid compounds which possess antifungal effect.

## CONCLUSION

In conclusion, this study clearly indicates the fact that SBN has the significant antifungal effect against *Candida* species when compared to HSP. These drugs could be good candidate to combine with regular antimicrobial agents. However, their exact role behind the antifungal effect is further warranted.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

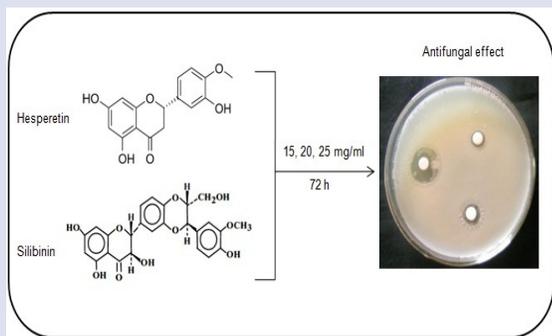
SBN: Silibinin; HSP: Hesperetin; CAS: Chemical Abstracts Service; AIDS: Acquired Immune Deficiency Syndrome.

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### GRAPHICAL ABSTRACT



### SUMMARY

In this study, we investigated the antifungal effects of silibinin and hesperetin against several fungal organisms. We found that silibinin has significant antifungal effect against *Candida* species when compared to hesperetin. Therefore, these drugs could be good candidates to combine with regular antimicrobial agents.

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