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In vitro antimicrobial activity of flavanoids of *Ocimum sanctum* with synergistic effect of their combined form

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ABSTRACT

Objective: To evaluate the antibacterial activity of flavanoids, Orientin and Vicenin, obtained from leaves of *Ocimum sanctum*, have also been compared by their combine sample. **Methods:** Aqueous extract of fresh leaves of *Ocimum sanctum* was assessed for the isolation and purification of different flavanoids. The antibacterial activity of Orientin, Vicenin and Combine sample of both these flavanoids was evaluated according to well diffusion method against some bacteria causing Urinary Tract Infection (UTI) in human. **Results:** The result indicated that the combined sample or synergistic activity of both individual flavanoids showed positive result against *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Staphylococcus cohnii* and *Klebsiella pneumonia* with zone of inhibition 20.12, 20.75, 20.95, 19.55 and 20.1 mm at concentration of 400 mg/ml respectively. But the individual flavanoids showed the positive result against only limited microorganism. **Conclusions:** The finding of the present study provides the evidence that this flavanoid sample is used as an antibacterial agent. This is also beneficial to use this combine sample of different flavanoids of *Ocimum sanctum* for medication and other purposes.

1. Introduction

Ocimum sanctum belongs to the family Lamiaceae and are used as an important component for the ayurvedic treatment of various diseases and also possess several pharmacological properties such as antifertility[1–2], anticancer, antidiabetic, antifungal, antimicrobial[3], hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions[4]. Various types of chemical compound such as phospholipids, glycolipids, steroids, carotenoids, phenolic compounds have been isolated from this plant.

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, they are found as plant pigments in a broad range of fruits and vegetables[5]. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been used for centuries to treat human disease[6]. Various important biological properties, including antibacterial activity were shown by the isolated flavonoids[7]. We chose

to study Orientin, Vicenin and their Combine sample for antibacterial activity and because no work on this flavanoid composition had been reported.

2. Materials and Methods

2.1 Extraction and Isolation

Fresh leaves of *Ocimum sanctum* were cleaned, dried at 36 °C for 5 days and pounded well. Ocimum flavanoids were isolated from the aqueous extract of fresh leaves, was prepared by refluxing the dried powdered leaf in double distillation water and concentrating by a method described somewhere else[8]. Isolation and purification of Ocimum flavanoids, Orientin and Vicenin were achieved by a method modified from Markham[9]. Combine sample of Ocimum flavanoids was prepared by mixing Orientin and Vicenin in equal proportion.

2.2 Antibacterial Assay

The test organisms included for study were gram positive *Staphylococcus aureus*, *Staphylococcus cohnii* and gram negative *Escherichia coli*, *Proteus* and *Klebsiella pneumonia*.

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The bacteria were grown in the nutrient broth at 37°C for 24 hours. 38.0 gm of Muller Hinton Media (Hi-Media) was mixed with 2 litre distilled water (pH 7.5) and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured in to petri dishes. The solidified plates were bored with the help of 5 mm diameter tip and these plates with wells were used for the antibacterial study. 1 gm of the each individual and combine sample of flavanoids were dissolved in DMSO (Dimethyl sulfoxide) separately and of 400 mg, 200 mg, 100 mg and 50 mg concentration of each extracts were tested against gram positive *Staphylococcus aureus* (*S. aureus*), *Staphylococcus cohnii* (*S. cohnii*) and gram negative *Escherichia coli* (*E. coli*), *Proteus* and *Klebsiella pneumoniae* (*Klebsiella*) for their antibacterial activity. It was demonstrated by well diffusion method^[10].

3. Result

The result of comparative antibacterial activity of different flavanoids (Orientin and Vicenin) and their combined form of *Ocimum sanctum* against *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Staphylococcus cohnii* and *Klebsiella pneumoniae* with different concentration (400, 200, 100 and 50 mg/ml) are shown in Figure 1, 2, 3 and 4. Orientin and Vicenin were active against limited microorganism. Orientin was found active against *Staphylococcus aureus*, *Staphylococcus cohnii* and *Klebsiella pneumoniae* in all concentration. Maximum zone of inhibition (18.04, 17.13 and 16.11 mm) in Orientin was observed in concentration of 400 mg/ml against *Staphylococcus aureus*, *Staphylococcus cohnii* and *Klebsiella pneumoniae* respectively. Vicenin was effective only against *Escherichia coli* and *Proteus*. Maximum zone of inhibition (18.84 and 17.16 mm) was also observed in same concentration as that of the Orientin, against *Escherichia coli*, *Proteus* respectively. The combination of both these flavanoids was found to be most active against all the bacterial strain. The highest zone of inhibition (20.12, 20.75, 20.95, 19.55 and 20.1 mm) was observed in concentration of 400 mg/ml against *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Staphylococcus cohnii* and *Klebsiella pneumoniae* respectively. The data shown by the combined sample (Figure 1, 2, 3, and 4) is due to the synergistic effect of both the above mentioned compounds.

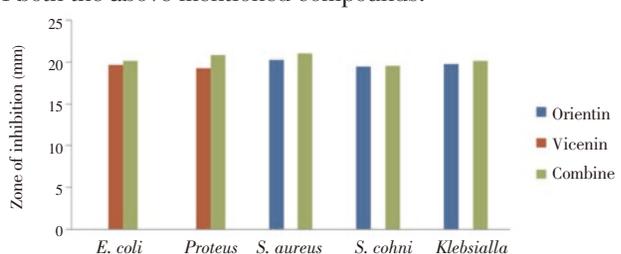


Figure 1: Showing Zone of inhibition at concentration 400 mg of combine and the individual extracts of the above mentioned plants against different bacterial strains.

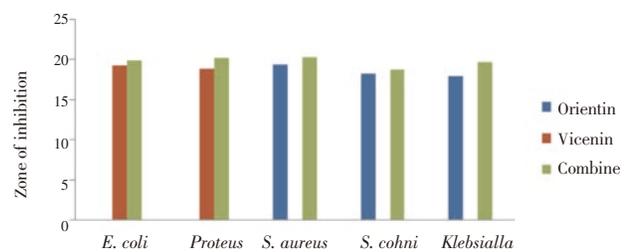


Figure 2: Showing Zone of inhibition at concentration 200 mg of combine and the individual extracts of the above mentioned plants against different bacterial strains.

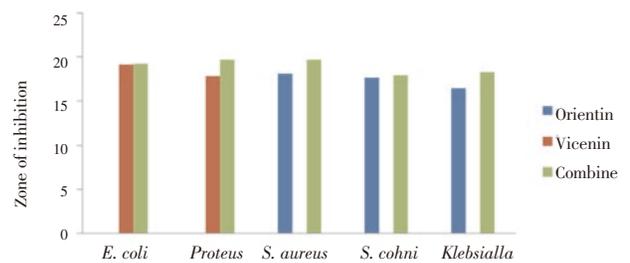


Figure 3: Showing Zone of inhibition at concentration 100 mg of combine and the individual extracts of the above mentioned plants against different bacterial strains.

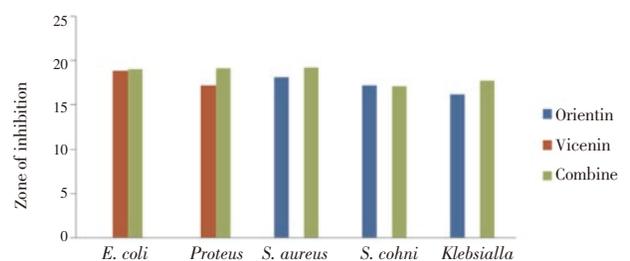


Figure 4: Showing Zone of inhibition at concentration 50 mg of combine and the individual extracts of the above mentioned plants against different bacterial strains.

4. Discussion

The ethanolic leaf extracts of *Catharanthus roseus*, *Lawsonia inermis* and *Chrysanthemum odoratum* showed least activity against *Staphylococcus aureus* when used separately. Whereas, the combination of these three plant-extracts exerted a higher activity^[11]. The ethanolic extract of *B. aegyptiaca* leaves in combination with other parts (stem bark, root bark and fruits) of *B. aegyptiaca* exerted synergistic effect against food-borne diarrheagenic bacterial^[12]. Similarly in this study combination of different flavanoids showed positive result against all microorganisms than the individual one. This result of antibacterial activity could probably be due to the synergistic effect of the Orientin and Vicenin which act upon the microbes and stand

out a good activity. The above studies strongly support the present finding.

5. Conclusion

From the result it has been observed that there is a possibility of using these flavanoids and their combination against bacteria causing Urinary Tract Infection (UTI) in human. The synergistic effect obtained in this case could lead to new choice for the treatment of infectious diseases. There is a scope to use this unique combination of Orientin and Vicenin flavanoids against UTI bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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