Antimicrobial Studies on Extracts of Four Species of Stachys

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Saeedi, et al.: Antimicrobial effect of Four Species of Stachys

The antimicrobial activity of the methanol extracts of the dried flowering aerial parts of Stachys byzantina, S. inflata, S. lavandulifolia and S. laxa (Labiateae) were studied using the disc diffusion method and determination of minimum inhibitory concentration (MIC) values against Staphylococcus aureus, Streptococcus sanguis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aspergillus niger and Candida albicans. The extracts of plants

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Staphylococcus aureus was stored in 4º. The extracts were evaporated to dryness at 40º, and each powder was extracted twice with methanol. Ground to fine powder. One hundred grams of the Resources of Mazandaran. Dried plant materials were 152, 154 and 155) were deposited in the Herbarium of Mazandaran. Botany, Research Center of Natural Resources of North of Iran and identified at the Department of from the suburb of Behshahr, Mazandaran province, The testing the antimicrobial activity. were determined against test microorganisms6. In studies were carried out by the disc diffusion method, extracts were dissolved the disc diffusion method, extracts were dissolved in methanol and applied to a 6 mm diameter paper disc. The extracts were tested at 10, 50, 100, 250, 500, 750 and 1000 μg/disc. Inhibition zone diameters were measured after 24 h. Gentamicin (50 μg/disc), amikacin (3 μg/disc) and amphotericin B (100 μg/disc, Sigma) were used as positive controls. MICs were determined by the dilution method at concentrations of 10 μg/ml to 25 mg/ml of culture medium. Gentamicin (2 mg/ml) and amphotericin B (100 μg/ml) were used as positive controls.

The yield of methanol extracts of Stachys byzantina, S. inflata, S. lavandulifolia and S. laxa was 14.1%, 14.3%, 10.1% and 10.6% w/w, respectively. Tables 1 and 2 gives a summary of the results of the antimicrobial effects and MICs of Stachys species investigated. The methanol extracts of the dried flowering aerial parts of S. byzantina, S. inflata, S. lavandulifolia and S. laxa exhibited concentration-dependent antibacterial activity against bacteria tested. The methanol extracts were more active against Gram-positive microorganisms (Streptococcus sanguis and Staphylococcus aureus). The extracts, however, did not show antifungal activity.

In 2004 and 2005, the antimicrobial activity of some endemic Stachys species including S. sivasica, S. anamurensis, S. cydnia, S. aleurites and S. pinardii was reported; the methanol extracts of Stachys L. were effective only against bacteria tested. In 2005, essential oils and ethanol extracts from the leaves and/or roots of 35 medicinal plants commonly used in Brazil were screened for antiCandida albicans activity; essential oils from 13 plants including S. byzantina showed anti-Candida activity; the ethanol extract was not effective at any of the concentrations tested.

In the present study, the results concluded that the methanol extracts of these plants have a potential as source of antibacterial agent of natural origin. Preliminary phytochemical studies showed that the aerial parts of the genus Stachys contain flavonoids. Flavonoids may be responsible for their antibacterial activity.

The sub cosmopolitan genus Stachys compromises more than 270 species and is justifiably considered as one of the largest genera of the Labiatae1. The genus Stachys includes 34 species in Iran2. Phytochemical investigations of Stachys species have shown the occurrence of flavonoids, diterpenes, phenyl ethanoid glycosides and saponins3. Plants of this genus have long been applied to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers4. S. byzantina C. Koch. (syn. S. lanata Jacq.), S. inflata Benth., S. lavandulifolia Vahl and S. laxa Boiss. and Buhse are aromatic plants, which grow in Azerbaijan, Golestan, Khorasan, Mazandaran and Tehran provinces of Iran5. A bibliographical survey showed that there were no reports on the antimicrobial activity of these species. In continuation of studies of Iranian species of the Labiatae family, we have had occasion to investigate the antimicrobial activity of S. byzantina, S. inflata, S. lavandulifolia and S. laxa.

The flowering aerial parts of S. byzantina, S. inflata, S. lavandulifolia and S. laxa were collected in May 2004 from the suburb of Behshahr, Mazandaran province, North of Iran and identified at the Department of Botany, Research Center of Natural Resources of Mazandaran. Voucher specimens (herbarium No. 151, 152, 154 and 155) were deposited in the Herbarium of the Department of Botany, Research Center of Natural Resources of Mazandaran. Dried plant materials were ground to fine powder. One hundred grams of the each powders were extracted twice with methanol. The extracts were evaporated to dryness at 40º, and stored in 4º.

Staphylococcus aureus PTCC 1112, Streptococcus sanguis PTCC 1449, Escherichia coli PTCC 1330, Pseudomonas aeruginosa PTC 1074, Klebsiella pneumoniae PTCC 1053, Aspergillus niger PTCC 5011 and Candida albicans PTCC 5027 were used for testing the antimicrobial activity. In vitro antimicrobial studies were carried out by the disc diffusion method and minimum inhibitory concentration (MIC) values were determined against test microorganisms6. In the disc diffusion method, extracts were dissolved

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Key words: Antimicrobial, extract, Stachys, MIC


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Determination of Ebastine in Pharmaceutical Formulations by HPLC

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Prabu, et al.: Determination of Ebastine by HPLC

A simple, precise and rapid RP-HPLC method was developed for the determination of ebastine in pharmaceutical formulations. The method was carried out on a Phenomenex RP-C18 column using a mixture of methanol and water (90:10) and detection was done at 262 nm. The linearity range was 5-100 \( \mu \)g/ml. The intra-day and inter-day precision were in the range of 0.22% to 0.49% and 0.24% to 0.73%, respectively.

Key words: Ebastine, reverse-phase liquid chromatography, solid dosage form

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Ebastine is a new generation of antihistamines which has potent and selective histamine H1-receptor antagonistic effect, but negligible anticholinergic and antiserotonergic properties. Ebastine is effective for the treatment of chronic idiopathic urticaria and allergic diseases with once daily regimen, and the antihistaminic action is mainly induced by the active metabolite, carebastine that is rapidly generated in the small intestine and in the liver.

To date, a couple of chromatographic methods have been reported to quantify ebastine and its metabolites in physiological sample. Kang et al and Rohatagi et al, recently improved the assay methodology for ebastine and carebastine by using a tandem mass spectrometry in human plasma. However, till date no assay procedure has been reported for the determination of this drug in pharmaceutical formulations. Hence, there is a need to develop a simple assay procedure for the determination of this drug in pharmaceutical formulations. The availability of an HPLC method with high sensitivity and selectivity would be very useful for the determination of ebastine in pharmaceutical dosage forms.

Ebastine (assigned purity 99.8%) was a gift sample from Eros Pharma, Bangalore, India. HPLC grade methanol and water were procured from Ranbaxy Fine Chemicals Limited, SAS Nagar, India and Qualigens Chemicals, India respectively. Commercially available ebastine tablets, claimed to contain 10 and 20 mg of ebastine, respectively, were procured from the local Pharmacy. Quantitative HPLC was performed on an isocratic high pressure liquid chromatograph (Shimadzu HPLC Class 10A Series) with two LC-10AT pumps, using a fixed wavelength guided by a programmable UV/Vis detector (SPD-10A). The column used was Phenomenex RP-C18 (250 mm × 4.6 mm i.d., Particle size 5 \( \mu \)m). The HPLC system was equipped with the software, Class LC-10AT series, version 5.03 (Shimadzu).

For HPLC, the mobile phase, methanol:water (90:10), was filtered before use through a 0.45 \( \mu \)m membrane filter. It was degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 ml/min. The run