SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME HETERO
BENZOCAINE DERIVATIVES

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ABSTRACT
The present study deals with condensation of urea, thiourea, semicarbazide and thiosemicarbazide with Ethyl-4-[(chloroacetyl) amino] benzoate under microwave irradiation in the presence of ethanol to afford two oxazole and thiazole derivatives. The synthesized compounds were characterized by spectral data such as IR, NMR; Mass. Compounds were screened for antimicrobial activity against strains of gram positive, and gram negative and fungal strains. All compounds showed good antibacterial and antifungal activity.

Keywords: Benzocaine, Oxazole, Thiazole, Antimicrobial, Urea, Thiourea, Semicarbazide, Thiosemicarbazide.

INTRODUCTION
Various heterocyclic compounds possess wide range of biological and pharmacological activities. Much attention has been paid during the last few decades to oxazole and thiazole moieties associated with a broad spectrum of activity such as antibacterial, antifungal, Pesticidal, insecticidal, anti-inflammatory, antitumor1, 3, 5, 9 etc., the demand for a new class of antimicrobial agents is substantially high in the last decade due to increased resistance towards various available antibiotics. An attempt has been made to synthesize two series of new oxazole and thiazole derivatives using microwave irradiation2, 7. Our present study deals with condensation of urea, thiourea, semicarbazide and thiosemicarbazide with Ethyl-4-[(chloroacetyl) amino] benzoate under microwave irradiation in the presence of ethanol to afford corresponding two oxazole and thiazole derivatives such as Ethyl-4-[(2-amino-1,3-oxazole-4-yl) amino] benzoate, Ethyl-4-[(2-hydrazinyl-1,3-thiazol-4-yl) amino] benzoate, Ethyl-4-[(2-amino-1,3-thiazol-4-yl) aminobenzoate, Ethyl-4-[(2-hydrazinyl-1,3-thiazol-4-yl) aminobenzoate. The synthesized compounds were characterized by spectral data such as IR, NMR, and mass spectrum data. The synthesized compounds were screened for antimicrobial activity against strains of gram positive (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, E.coli) and fungal strains (Aspergillus niger, Aspergillus fumigates). All compounds showed good antibacterial and antifungal activity.

MATERIALS AND METHOD
Melting point of the synthesized compounds were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on ABB BUMEM FTIR Spectrophotometer with KBr pellets. H1 NMR Spectra was recorded on Burker AV 400MHz and Mass Spectra were recorded on GCMS QD 5000 Shimadzu. The test compounds were synthesised by the following procedure.

Synthesis of ethyl-4-[(chloroacetyl) amino] benzoate
Ethyl 4 amino benzoate (0.01 mole) in 120ml of ethanol was shaken in a magnetic stirrer for 1½ hours. Chloroacetyl chloride (0.01 mole) was added in drops to the above mixture. The mixture was then allowed to be stirred for 1 hour. The stirred mixture was then refluxed for 2 hours. The mixture was then poured into ice cold water. The mass obtained was filtered and recrystallized with ethanol. The solid mass was recrystallized using ethanol to afford 70% yield. Rf = 0.8, Melting Point 140-142ºC.

Scheme I

Synthesis of Compound I: Ethyl-4-[(2-amino-1, 3-oxazole-4-yl) amino] benzoate
Ethyl-4-[(chloroacetyl) amino] benzoate (0.01 mole) and Urea (0.01mole) were dissolved in ethanol; the mixture was irradiated in microwave oven at a low power for 15 minutes. The solid mass was recrystallized using ethanol to afford 70% yield. Rf = 0.8, Melting Point 220-224ºC.

IR (KBr cm-1): 3488(NH Str), 1713(C=O), 1643(C=N), 1360 (C-O), 2835(C-H Aro str)
NMR: δ 2.78 (CH3), δ 7.9 (CH), δ 8.2 (NH), δ 9.2 (NH2)
m/z: 248.10

Synthesis of Compound II: Ethyl-4-[(2-amino-1, 3-thiazol-4-yl) amino] benzoate
Ethyl-4-[(chboroacetyl) amino] benzoate (0.01mole) and Thiourea (0.01mole) were dissolved in ethanol; the mixture was irradiated in microwave oven at a low power for 15 minutes. The solid mass was recrystallized using ethanol to afford 72% yield. Rf = 0.8, Melting Point 198-202ºC.
IR (KBr cm-1): 3273(NH Str), 1619(C=O), 2865(C-H Aro str), 730(C=O)
NMR: δ 6.54 (NH), δ 7.3 (CH) (oxazole), δ 6.27 (CH3), 6.72-6.8 (Ar H)
m/z: 248.10

Synthesis of Compound III: Ethyl-4-[(2-hydrazinyl-1, 3-thiazol-4-yl) amino] benzoate
Ethyl-4-[(2-hydrazinyl-1, 3-oxazol-4-yl) amino] benzoate, Ethyl-4-[(2-hydrazinyl-1, 3-thiazol-4-yl) amino] benzoate. The synthesized compounds were characterized by spectral data such as IR, NMR, and mass spectrum data. The synthesized compounds were screened for antimicrobial activity against strains of gram positive (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, E.coli) and fungal strains (Aspergillus niger, Aspergillus fumigates). All compounds showed good antibacterial and antifungal activity.

Scheme II

Synthesis of Compound III: Ethyl-4-[(2-hydrazinyl-1, 3-thiazol-4-yl) amino] benzoate
Ethyl-4-[(2-hydrazinyl-1, 3-oxazol-4-yl) amino] benzoate (0.01mole) and Thiourea (0.01mole) were dissolved in ethanol; the mixture was irradiated in microwave oven at a low power for 15 minutes. The solid mass was recrystallized using ethanol to afford 80% yield. Rf = 0.8, Melting Point 198-202ºC.
IR (KBr cm-1): 3495(NH Str), 1685(C=O), 1363 (C-O), 2995(C-H Aro str)
NMR: δ 6.54 (NH), δ 7.3 (CH) (oxazole), δ 6.27 (CH3), 6.72-6.8 (Ar H)
m/z: 248.10

Synthesis of Compound IV: Ethyl-4-[(2-hydrazinyl-1, 3-thiazol-4-yl) amino] benzoate
Ethyl-4-[(2-hydrazinyl-1, 3-thiazol-4-yl) amino] benzoate (0.01mole) and Thiourea (0.01mole) were dissolved in ethanol; the mixture was irradiated in microwave oven at a low power for 15 minutes. The solid mass was recrystallized using ethanol to afford 72% yield. Rf = 0.8, Melting Point 198-202ºC.
IR (KBr cm-1): 3431(NH Str), 1685(C=O), 1363 (C-O), 2995(C-H Aro str)
NMR: δ 6.54 (NH), δ 7.3 (CH) (oxazole), δ 6.27 (CH3)
MASS: C₁₂H₁₅N₄O₃, m/z: 263.7

Fig. 1: Synthesis of Benzocaine Derivatives

Antimicrobial Activity

The synthesized compounds were subjected to antimicrobial activity. Antimicrobial activities were observed for all compounds using strains of gram positive such as (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, E.coli) and fungal strains (Aspergillus niger, Aspergillus fumigates).

The antimicrobial activities of the synthesized compounds were studied by disc diffusion method. Bacterial inoculums were spread on Nutrient agar. After the inoculums dried 6 mm diameter wells were made in the agar plate with a sterile cork borer. The synthesized compounds were dissolved in DMF at concentrations of 10 μg, 20 μg, 30 μg per ml. Ciprofloxacin 50 μg/ml was used as standard for the antibacterial activity and Ketoconazole was used as standard for the antifungal activity. The Petri plates were incubated at 37°C for 24 hours. The Zone of inhibition was measured in mm to estimate the potency of the test compounds.

<table>
<thead>
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<th>Organism</th>
<th>Staphylococcus aureus (μg/ml)</th>
<th>Bacillus cereus (μg/ml)</th>
<th>Escherichia Coli (μg/ml)</th>
<th>Pseudomonas aeruginosa (μg/ml)</th>
<th>Aspergillus niger (μg/ml)</th>
<th>Aspergillus fumigates (μg/ml)</th>
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<tr>
<td><strong>Compound</strong></td>
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<td><strong>30</strong></td>
<td><strong>Std</strong></td>
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<tr>
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</table>
RESULTS AND DISCUSSION

The synthesized compounds were characterized through IR, H NMR and mass spectra. All synthesized compounds in the present study showed expected characteristic absorption bands for phenolic OH, NH, C=N, C-O, C-H, C-S. The investigation of antimicrobial screening data revealed that all the tested compounds shown good antimicrobial activity.

CONCLUSION

The synthesized compounds were subjected to antimicrobial activity. Concentrations of 10μg, 20μg, 30μg per ml were screened for antimicrobial activity. Among the synthesized compounds 30 μg/ml concentration of all synthesised compounds showed good antimicrobial activity against gram positive such as (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, E.coli) and fungal strains (Aspergillus niger, Aspergillus fumigates and comparatively potent than standard.

REFERENCES