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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS OF *AGERATUM CONYZOIDES* (ASTERACEAE)

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Abstract

Essential oils from different organs of *Ageratum conyzoides* obtained by hydro distillation were analyzed through Gas Chromatography and Gas Chromatography/Mass Spectrometry. The essential oils were tested for antibacterial activity by the Disc-diffusion assay and antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms in millimeters. The samples were active against *Staphylococcus aureus* and *Staphylococcus pyogenes*, with zone of inhibition 4.0 and 3.9 mm respectively while the inhibition values for streptomycin varied from 2.6 to 3.7 mm. Streptomycin was used as a positive control. From the Gas Chromatography/Mass Spectrometry results, the compounds found were 3-phenylisoquinoline and its isomers.

Keywords: *Ageratum conyzoides*; essential oil composition; antibacterial activity.

Introduction

Essential oils are important constituents of some higher plants comprising monoterpenes, sesquiterpenes, arylpropanoids, and other derivatives. The antimicrobial properties of essential oils have been recognized long ago and they have been scientifically established (S.G. Deans and G. Ritchie.1987). The family Asteraceae includes about 25000 cosmopolitan species, many of which are rich in essential oil producers with biological activity (A.L. Okunade.2002). The genus *Ageratum* belongs to the Eupatoriae tribe and many of its species, such as *Ageratum conyzoides*, has been chemically studied (A.L. Okunade.2002, V.S. Rana and M.A. Blazquez.2003, R.H.C. Nébié *et al.*2004). *Ageratum conyzoides* is a plant found in North Ethiopia (T. Desta *et al.*2014). The methanolic extracts of *Ageratum conyzoides* have identified

toxic pyrrolizidine alkaloids (T. Desta *et al.* 2014). This species presented antimicrobial activities and the leaves have been used in folk medicine to treat inflammation, infections and as analgesic (Kamboj A, S. A. 2008).

Until the present there is no study of the chemical constituents and antibacterial activity of the *Ageratum conyzoides* essential oils from Ethiopia. In this research, the composition of the essential oils from leaves, stems, and seeds of *Ageratum conyzoides* and their antibacterial tests will be described.

Materials and Methods

1. Plant Material

The biomass of the plant, *Ageratum conyzoides* consisting of stems, leaves and seeds were collected during the month of November 2013 from the veno-occlusive disease (VOD) affected areas of North Ethiopia. The plant was identified by the Botanist of Addis Ababa University and a Herbarium sheet (No. 01) was deposited at the National Herbarium of Addis Ababa University, Ethiopia.

2. Isolation of the Essential Oils

The shade air dried plant leaves, stems and seeds of *Ageratum conyzoides* (500 g) were introduced in to conical flask with 500ml of water and it was hydro distilled in a Clevenger type apparatus (E.F.K. Denny. 1989). After 4 h of distillation, the essential oils were removed from the surface of the water. The oils were dried over anhydrous sodium sulphate and kept in sealed glass vials in the refrigerator at 4⁰C until analysis and tests.

3. Gas chromatography and Gas chromatography/mass spectrometry analysis (GC/MS)

Capillary gas chromatography was performed using a Hewlett-Packard 6890 gas chromatograph; fused silica capillary column HP-5 (5% biphenyl and 95% dimethylpolysiloxane, 60m x 0.25 mm, 0.25 µm film thickness); helium as carrier gas (1 mL/min); and temperature programming from 70 to 290°C (2°C/min); injector temperature 270°C and detector temperature 300°C.

The GC/MS analysis of the oils were performed on a Hewlett Packard series 6890 gas chromatograph coupled to MS HP5972 mass spectrometer under the following analytical conditions: HP5MS column (30 m x 0.25 mm x 0.25 µm film thickness); helium (1 mL/min); programmed temperature 60°-240°C (3°C/min); injector temperature (260°C) and interface (200°C); ionization energy, 70eV; scan range, 200-300 amu; scan time, 1s. Compound identification was based on the

comparison of retention indices, mass spectra and the NIST spectrometer data bank, as well as comparison with literature data (R.P. Adams. 2007).

4. Microbial strains

The essential oils of *Ageratum conyzoides* were tested against a group of microorganisms including *Staphylococcus aureus*, *Staphylococcus pyogenes* and *Pseudomonas aeruginosa*. These microorganisms, belonging to the American Type Culture Collection (ATCC), were supplied by the Bangalore test house, India. The antimicrobial activity was tested through disk-diffusion assay and Streptomycin was used as a positive controls.

Results and Discussion

The shade dried plant parts of *Ageratum conyzoides* found to contain 0.25% of essential oil for leaves, stems and seeds totally. Three compounds were identified in the essential oil from both leaves; stems and seeds and also they are isomers (Table1). The compounds identified in the essential oils from *Ageratum conyzoides* belong to phenylisoquinoline series. The oil was Characterized by the abundance 3-phenylisoquinoline (100%), it was observed that 3-phenylisoquinoline had a significant presence in the oils analyzed, thus being a true chemical marker for the *Ageratum conyzoides* essential oil.

Table-1: The component of essential oils from *Ageratum conyzoides*.

Peak no	Compound	RT(min)	Peak height	%peak area
1	3-phenylisoquinoline	32.186	167	15.131
2	3-phenylisoquinoline	32.439	158	29.038
3	3-phenylisoquinoline	32.481	174	55.830

Antibacterial activity of essential oil of *Ageratum conyzoides*

Disc-diffusion assay

The essential oil was dissolved in methylene chloride solvent to a final concentration of 30mgmL⁻¹ and sterilized by filtration through Millipore filters. Antimicrobial tests were then carried out by the disc diffusion method (Murray et al.,1995) using 100 µL of suspension containing 108 CFUmL⁻¹ of bacteria spread on nutrient agar (NA).The discs (6mm

in diameter) were impregnated with 20 µl of essential oil and placed on the inoculated agar. Negative controls were prepared by using same solvents that were employed to dissolve the essential oil. Streptomycin was used as a positive reference standard to determine the sensitivity of one strain in each microbial species tested. The inoculated plates were incubated at 37°C 24 h with clinical bacteria strains. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms in millimeters and the results were tabulated in Table -2.

Statistical methods

All the data were expressed as mean ± standard deviation (SD). Statistical significance was performed by analysis of variance (ANOVA), followed by Bonferroni's test; $p < 0.05$ was considered to be significant.

Table-2: Antibacterial activity of essential oil of *Ageratum conyzoides* by disc diffusion method.

S.No	Test microorganism	Essential oil DD(mm)	Control DD(mm)
1	<i>Staphylococcus aureus</i>	4.0	3.7
2	<i>Staphylococcus pyogenes</i>	3.9	2.6
3	<i>Pseudomonas aeruginosa</i>	2.8	2.3

The antibacterial activity of essential oil of *Ageratum conyzoides* against the microorganisms examined in this study and its potency were examined by the presence and absence of inhibition of zones. Streptomycin was used as a positive control. The results are given in Table-2. The data showed that the essential oil of *Ageratum conyzoides* has a good antibacterial activity of the bacteria tested. It is active against *S.aureus* and *S.pyogenes*, with zone of inhibition 4.0 and 3.9 mm respectively while the inhibition values for streptomycin varied from 2.6 to 3.7 mm. This results showed that the essential oil of *Ageratum conyzoides* support to the ethno therapeutic claims for treating skin diseases (Venkatesan *et al.* 2005) as *S.aureus* and *S. pyogenes* are primary causative agents of skin and soft tissue infections. The antibacterial activity of the oil of *Ageratum conyzoides* may be attributed to the interaction of the ingredients of the oil with the substances.

Conclusions

Three essential oil compounds were isolated from *Ageratum conyzoides* and both are isomers namely 3-phenylisoquinoline, has not been described previously from *Ageratum conyzoides* and it was reported for the first time.

The chemical structures of the isolated compounds have been elucidated using GC-MS. The essential oil of *Ageratum conyzoides* is a potential candidate to be used as an antibacterial agent in drugs for the treatment of infectious diseases. To the best of our knowledge, this is the first report of the essential oils of *Ageratum conyzoides* from Ethiopia.

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