ANTIMICROBIAL ALKALOIDS FROM EUPHORBIA THYMIFOLIA

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Considerable amount of work has been reported on alkaloids having antimicrobial properties. That emetine is the principle in Ipecac active against the intestinal parasite, *Entamoeba histolytica*, was amply demonstrated by Vedder.[1] Extracts of cinchona cured the human beings infected with malaria parasites, and quinine, the curative agent was isolated by Pelletier and Caventon in 1820. The alkaloid, umbellatine was isolated by Chatterjee[2] in 1940 from two Himalyan species of Berberis and was found to inhibit the growth of *Lishmania tropica* and *paramecium*. Jennings[3] showed that gindricine which was isolated by Siddiqui in 1943 from the plant, *S. glabra*, inhibited the growth of *Staph. aureus*, *Stretococcus pyogenes*. Salam and Ahmad[4] showed that glycosine from *Glycosmis pentaphylla* was active against *B. cereus*, *B. subtilis*, *Staph. aureus*, *Proteus vulgaris*.

In the present investigation the active principles of the plant, *Euphorbia thymifolia*, have been isolated and their anti-bacterial properties have been studied. Though Kartikar and Basu[5] reported that this plant contains an alkaloid like principle, no attempt has so far been made on the isolation of the alkaloid.

Experimental

Fresh plants of *Euphorbia thymifolia* were collected, washed and dried in the sunlight and powdered. The dried powdered plants were extracted with 1.5 percent cold hydrochloric acid, by soaking for about 24 hours, Liquor ammonia was added to the extent till alkaline and precipitate separated out.

The precipitate was allowed to stand and the clear liquid siphoned off (A). The precipitate was washed with water and then repeatedly extracted with chloroform, till the residue was free from alkaloid. The chloroform extract was then concentrated at low temperature (50°C.). This was designated as fraction 'a'.

After complete extraction with the above solvent, the precipitate was further extracted with amyl alcohol. The alcoholic extract was concentrated and tested for the presence of alkaloid and a second fraction containing alkaloid was obtained. This was designated as fraction 'b'.

To the decanted clear liquid (A), 2N sodium hydroxide solution was added when brown precipitate was obtained; the precipitates were allowed to stand. The upper clear liquid was decanted off and the precipitates extracted with chloroform. The chloroform extract was concentrated and the concentrate gave positive test for alkaloid. This was designated as fraction 'c'.

Paper Chromatography.—Whatman paper no. 1 was soaked with M/15 phosphate buffer and air-dried. The three different fractions (i.e. a, b and c) were spotted by a micropipette on the paper strip on a line 7 cm. from the end of the strip. The paper was developed in the solvent system of *n*-butanol, formic acid and water (10:1:10). The dried paper was sprayed with the modified Dragenderff's reagent. Three spots of different Rf values showed three different alkaloids in the three fractions.

Antagonistic Property of the Fractions.—Organisms used for the investigations were:- (1) *E. coli*; (2) *B. subtilis*; (3) *B. cereus*; (4) *Staph. aureus*.

Solid residues of fractions 'a' and 'c' were dissolved in chloroform and that of fraction 'b' was dissolved in amyl alcohol.

Filter papers were cut into small discs of about 0.5 cm. diameter and sterilised. Twelve such sterilized paper discs were taken and to each four discs was added one drop of each fraction by a sterilized dropper. The discs were air-dried before use.

Four test tubes, each containing 15 ml. of the sterilized nutrient agar, were taken and inoculated with young culture of the test organisms and then plated. When the agar solidified, the paper discs containing 'a', 'b' and 'c' were placed carefully on the agar at 3 corners of the plate. The petri-dishes were kept in the refrigerator overnight for diffusion of the substance in the media. They were then placed in the incubator at 30°C. for 12 hours. The organism multiplied there and the action of the fraction on them was noted. Formation of clear zones showed inhibition of growth and the diameter of the inhibition zone was recorded in millimeter (Table 1).

TABLE 1.

Test organism	Activity of 'a' Diameter of inhibition zones	Activity of 'b' Diameter of inhibition zones	Activity of 'c' Diameter of inhibition zones
E. coli	11 mm.	0 (-Ve)	9 mm.
B. subtilis	20 mm.	0 (-Ve)	5 mm.
B. cereus	0 (-Ve)	0 (-Ve)	0 (-Ve)
Staph. aureus	0 (-Ve)	0 (-Ve)	0 (-Ve)

Results

Of the three fractions, 'a' was comparatively more active.

4 kg. of the dry materials were extracted as described and 0.2 g. material of the fraction 'a' was collected. Cube-like crystals were obtained by slow evaporation of the alcoholic solution of this fraction. The pure crystals melted at 187°C. Chromatographic studies showed that the crystals are single substance. The crystals were active against *B. subtilis* and *E. coli*.

Chemical investigations of the crystalline product:-

The crystalline product was found to contain active unsaturation and secondary amine group. Its molecular weight was determined by Rast's method and was found to be 418. The substance is fairly soluble in ethyl alcohol and chloroform and insoluble in water, ether, benzene, petroleum ether and carbon tetrachloride.

Further work is in progress.

References

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COMPOSITION OF OIL FROM THE SEEDS OF CUCUMIS PROPHETARUM

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The work on *Cucumis prophetarum* was taken up in order to examine its alkaloidal contents. The alkaloidal content was not appreciable, but in the course of the investigations, it was found that the seeds contained oil in considerable quantity, that is 28.4 percent. The composition of the oil was therefore investigated. The dry seed content by weight of the original fruit was found to be 5.2 percent.

Extraction of Oil.—The seeds were crushed and extracted exhaustively with petroleum ether (b.p. 40—60°) in a soxhlet extractor. The oil was analysed for density, refractive index, acid value, iodine value, saponification value, unsaponifiable matter, peroxide value, diene value and hydroxyl value by the usual methods. The meal was analysed for protein and ash contents. The results are given in the following Tables:

PHYSICO-CHEMICAL CHARACTERISTICS OF THE OIL.

Specific gravity at 33°C.	0.9174
Refractive index at 25°C.	1.4711
Acid value	11.6
Iodine value (Hanus)	133.0
Saponification value	186.8
Unsaponifiable matter	1.4
Peroxide value	20.9
Diene value	0.63
Hydroxyl value	8.7

CHARACTERISTICS OF THE MEAL.

Percentage yield (by weight of seeds)	71.6
Protein	25.6%
Ash	6.5%

Resolution into Acid Fractions.—The oil was saponified by refluxing with 0.5 N alcoholic potassium hydroxide. After removal of alcohol, aqueous solution of soap was prepared and extracted with diethyl ether to remove unsaponifiable matter. Fatty acids were obtained by acidification of soap solution and separated into fractions by Twitchells lead salt alcohol method. The solid acid fraction was found to be 17.6 percent, trans acids being 7.0 percent and saturated acids, 10.6 percent.