CHEMICAL AND PHARMACOLOGICAL STUDIES OF THE PLANT ERVATAMIA CRISPA (syn. TABERNAEMONTANA-CRISPA-APOCYANACEAE)

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ABSTRACT

Chemical and Pharmacological studies have been made on the extracts of the plant Ervatamia Crispa (Apocyanaceae). Three compounds were obtained in pure condition whose analytical data are furnished. Pharmacologial activity has been found in two partially purified extracts.

INTRODUCTION

ERVATAMIA Crispa (syn. Tabernaemontana Crispa) (Fam. Apocyanaceae) is an under tree growing wildly in many parts of Kerala and Tamil Nadu. The existence of considerable amounts of alkaloids in this plant was first reported[1] in 1952 and reference to literature revealed that no further work has been done on this. Many medicinal properties have been ascribed to the plants of the Ervatamia (Tabernaemontana) group and since no pharmacological work has been done on this particular plant, a systematic pharmacological screening of the drugs obtained was taken up.

EXPERIMENTAL

The dry root bark powder R (1130 g) obtained from mature plants collected from Trichur, Kerala State was first extracted with boiling petroleum ether (60–80°). The petroleum ether extract on evaporation gave 53 g of the solid residue (marked A). The defatted root powder was digested with 1% hydrochloric acid and the acid solution was separated by filtration. The acid solution was then treated with excess of liquor ammonia until the solution was basic and extracted with chloroform. The chloroform extract was evaporated to remove the solvent leaving residue B (27 g). When the solid residue (A) was digested with 1% HCl and the acid solution was treated with ammonia and then extracted with chloroform and the chloroform solution evaporated, it gave another solid mixture (C) (7 g). Thus altogether the plant material contained about 34 g of basic material (alkaloidal) which is very significant.

Column chromatography of the basic fractions was carried out on neutral alumina eluting with (1) petroleum ether (60–80°), (2) benzene, (3) ethyl acetate, (4) chloroform, (5) chloroform ethanol (9:1), (6) absolute ethanol.

The following five main fractions were obtained from B. (1) petroleum ether eluate, (2) benzene eluate, (3) ethyl acetate eluate, (4) chloroform:ethanol (9:1) eluate, (5) absolute alcohol eluate. Mixture C however gave residues only from the first three eluates.

TLC monitoring of the eluates revealed that the *petroleum ether eluate* contained two components. These were successfully separated by column chromatography on silica gel G using chloroform as eluent. The first component RF value of 0.75 is named TY and the second compound RF 0.20 as TZ (silica gel G adsorbent and chloroform solvent for both). The benzene eluate showed the presence of 4 components on TLC. The ethyl acetate eluate after repeated purification and final separation using silica gel and chloroform:ethanol (9:1) as eluent gave another pure compound TX (see later). TX is one of the major substances present in the plant.

All the three samples gave positive tests for alkaloids with the alkaloidal reagents. Other data are as follows:

Compound TY crystallised from petroleum ether as colourless crystals. m.p. 134–135° (Uncorrected). Mol. wt. by Rast's method 362.9. $[\alpha]_D^{24.5°}$ chloroform $-26.73°$ m.p. of chloroplatinate 131° m.p. of picrate 115° UV Ethanol max. nm. 226, 285, 293. IR. CH_2Cl_2 3450 (NH)

2900 (CH_2) 1740 and 1660 cm^{-1} (CO)

NMR ($CDCl_3$) showed aromatic protons at 7.3 ppm. Methoxyl at 3.7 ppm, triplet at 0.9 ppm and multiplets from 1.2 to 3.4 ppm.

The mass spectrum gave the fragmentation patterns at M/E 122, 124, 136, 154, 167, 184, 186 and 214. Higher fragments could not be obtained.

Compound TZ crystallised from petroleum ether as colourless crystals. m.p. 126–127° (Uncorrected). Mol. wt. by Rasts' method 380. $[\alpha]_D^{24.5°}$. Chloroform $-36.42°$ m.p. of chloroplatinate 154–156° m.p. of picrate 171° (Decomp) UV Ethanol λ max. nm 225, 284, 292. IR CH_2Cl_2 at 3450 (NH) 3400–3250 (Broad) 1080 (OH) 1739, 1660 cm^{-1} (CO)

NMR ($CDCl_3$) Aromatic protons 7.3 ppm.

Methoxy at 3.7 ppm. Broad doublet at 0.95 ppm and multiplets from 1.2 to 3.4 ppm.

Compound TX: RF 0.95 (Silica gel G and chloroform:ethanol 9:1 developer) it was obtained as a brown powder from methanol. m.p. 169–170° (Uncorrected). Mol. wt. by Rasts' method 659.2 $[\alpha]_D^{24.5°}$ chloroform + 33.17° m.p. of chloroplatinate 138° m.p. of picrate 215° m.p. of dinitro-benzoate 138° UV Ethanol λ max. nm 225, 281, 292.

IR CH_2Cl_2 at 3450 (NH) 2860 (Broad) 1720, 1680 cm^{-1} (CO). Mass spectra—same type of fragmentation as TY. Higher fragments could not be obtained.

Three other components were also obtained in a homogeneous condition, one from benzene eluate and two more from chloroform:ethanol (9:1) eluates but they could not be further characterised. It appears that the plant material contains at least 9 components as shown by the TLC study of the partially purified samples.

Pharmacological Studies

The following pharmacological studies have been made on the two extracts A and B. (a) Preliminary screening in mice and rats. (b) Analgesic study (Gujral, M. L. and G. Khanna (1956) in Wistar Albino rats. (c) Anti-convulsant activity with Wistar Albino rats. (d) Effect on skeletal muscle frog rectus abdominus muscle. (e) Effect on smooth muscle—Guineapig ileum—as per the method described by the Staff, Department of Pharmacology, Edinburg University (1968). (f) Effect on Cardiovascular system of Dog (Jackson, D. E., 1939).

Both fractions have no significant effect on (i) autonomic and behavioural changes; (ii) analgesic studies and (iii) anti-convulsant action. Extract A possesses nicotine-like effect producing skeletal muscle contraction ganglion stimulation in small doses and ganglion blockade in large doses. Extract B possesses skeletal muscle relaxing property, minimal muscarinic blocking effect and negligible ganglion blocking effect and histamine releasing property.

Acute toxicity studies have given LD_{50} of the extract A as 89.15 mg/kg and in the case of Extract B there is no mortality of the experimental animals even upto a dose of 500 mg/kg.

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PHENOLIC ACIDS AS POTENTIAL INHIBITORS OF PLANT AMYLASE

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Abstract

Plant amylase derived from two sources (*Lens culinaris* and *Phaseolus aureus*) was inhibited *in vitro* by salicylic acid, caffeic acid and gallic acid. There was total inhibition at 50 mM concentration of the inhibitors. The inhibition was of non-competitive type with an apparent K_i values as 14, 8 and 8.5 mM for salicylic acid, caffeic acid and gallic acid respectively.

Introduction

PHENOLIC acids are known to act as analogues of growth hormones[1,2] and influence many enzyme systems[3,4]. Amylases occur widely in germinating seeds and play an important role during germination. The interaction of phenolic compounds with amylases was, therefore, considered to be of physiological significance. This paper deals with the *in vitro* interaction of caffeic acid, gallic acid and salicylic acid with mungbean and lentil seed amylase.

Materials and Methods

The enzyme preparation was carried out as described previously[5]. The seeds of local cultivars of