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SOME PROPERTIES OF VARIOUS FRACTIONS OF THE SEEDS OF ARISTOLOCHIA BRACTEATA

(With One Table)

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بعض خواص الأجزاء المختلفة لبذور نبات عرق العقرب

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في هذه الدراسة تم اجراء كروماتوقرافيا الورق للأجزاء المختلفة لبذور نبات أم جلاجل (عرق العقرب) وتم تسجيل قيم معامل العرقلة وتعريض النقاط للأشعة فوق البنفسجية. اعطى المستخلص الكحولي خمسة نقاط أربعة منها صفراء اللون تمت معاينتها بالأشعة فوق البنفسجية اضافة إلى منطقة واحدة ذات اشعاع أزرق هذه النتيجة اشارت إلى وجود قلويد. تبخر الايثر اعطى راسب حمضي اصفر اللون ثبت احتواءه على مركبين غير قلويديين. تم الكشف عن ثلاثة قلويدات في كلوريد القلويد الرباعي.

SUMMARY

Paper chromatography of different fractions of the seeds of *A. bracteata* was carried out. The Rf values were recorded and the spots were exposed to U.V. light. The alcohol extract gave five spots. Four yellow spots were seen in U.V. light, and one spot fluorescing blue in the same U.V. light. This result indicated the presence of alkaloid. Evaporation of the ether gave a yellow acidic residue which was found to contain two non-alkaloidal compounds. Three alkaloids were detected in the quaternary alkaloid chloride.

Key words: *Paper chromatography, aristolochia bracteata, alkaloids. Rf values.*

INTRODUCTION

Aristolochia species (Aristolochiaceae) are recorded in a list of plants used in Kenya, Ethiopia, Tanzania and Sudan for the treatment of infestation with nematodes. Worldwide, *A. bracteata* is used against

snake bites and scorpion stings (Morton, 1975; Hazlett, 1986; Houghton and Osibogun, 1993; Coe and Anderson, (1996).

Aristolochia trilobata is used for stomach pain and its leaf tinctures are used for the treatment of diarrhoea (Giron *et al,* 1991). It has been shown that aristolochic acid inhibits inflammation induced by immune complexes and non-immunological agents (Moreno, 1993). Moreover, aristolochic acid inhibits the activity of snake venom phospholipase (PLA_2) by forming 1:1 complex (Lans, 2001).

In the Sudan, *Aristolochia bracterata,* locally known as Um Galagel or Erg Elagrab, is widely distributed in central, eastern and western areas and is used as antidote to scorpion bites and as an anthelmintic (Brown and Massey, 1929).

Chemical constitution of toxic substances in plants is important. Alkaloids produce varying degrees of physiological reactions when introduced into animals (Schaoental, 1963, 1970, Bull *et al.,* 1968). There is, as yet, little information available regarding the toxic properties of poisonous plants in the Sudan.

In our previous repot, (Barakat, *et al.*, 1983) we have shown the toxicity of *A. bracteata* in Nubian goats. In addition the combined toxicity of *A. bracteata* and Rotundifolia to Nubian goats was investigated (Eldirdiri, *et al.*, 1987).

Plants of the genus Aristolochus such as *A. clematis* and *A.densivenia* contain an alkaloid, aristolochine and probably other substances (Watt and Breyer-Brandwijk, 1962; Clarke and Clarke, 1967). It has been found that *A. bracteata* contains Aristolochic acid, fixed oils and an orange yellow compound (Watt and Breyer-Brand wijk, 1962).

Aristolochia Bracteata is used in traditional medicines as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Lans, *et al.,* 2001).

Antibacterial activity of dried extracts of roots of *A. bracteata* was evaluated against a few gram-positive and gram-negative bacteria. All the erude extracts showed a broad spectrum of antibacterial activity (Negi *et al.,* 2003).

El Tahir *et al.,* (1999) investigated the antiplasmodial activity of extracts from *Gardenia lutea, cassia tora, Acacia nilotica* and *Aristolochia bracteolate.*

The ethanol extract of the shade-dried leaves of *Aristolochia bracteolate* was studied for its effect on wound healing in rats. The plant showed a definite positive effect on wound healing, with a significant

increase of the level of two powerful antioxidant enzymes (Shirwaikar, *et al.*, 2003).

The Sudanese species *A. bracteata* is of considerable interest. There are no reports on the chemical constituents of this species. The purpose of the present preliminary investigation was to throw light on some properties of the different fractions of the fully-ripe seeds of *A. bracteata.*

MATERIALS and METHODS

Preparation of Various Extracts of Aristolochia Seeds:

A sample of 500g of Aristolochia seeds was dried in the sun, finely ground in a mortar, defatted with petroleum ether and extracted with 95 percent alcohol. The extractives were then fractionated. The petroleum ether was evaporated in vacuum to give a residue (Fraction I). The defatted seeds were then extracted with 95% alcohol. The alcohol extract was concentrated to a thick syrup which was poured slowly with continuous stirring into warm diluted HCI at pH2. The mixture was refrigerated overnight and the supernatant was decanted. The decantate was concentrated, rendered alkaline with ammonia and extracted with ether. The ethereal extract was evaporated to give a residue (Fraction II).

The remaining aqueous alkaline solution was extracted with methylene chloride and evaporated to give a residue (Fraction III). The aqueous alkaline solution was then acidified (pH2) and treated with a freshly prepared saturated aqueous solution of ammonia reineckate. The reineckates were converted to chloride by means of HC1.

Paper chromatography:

Paper chromatography of the different fractions of the seeds of A. bracteata was carried out using the solvent system B.A.W. (butanol, ammonia and water, 50-8-1) and B.Ac.W. (butanol, acetic acid and water, 5-1-4). The Rf values were recorded and the spots were exposed to ultra-violet light.

Preparation of Dragendorff's reagent:

Preparation of Dragendorff's reagent is the most commonly used reagent for alkaloids which appear as orange spots on the lighter to orange to yellow background. The limit of detectability with Dragendorff's reagent lies usually between 3 and 10 µg>Dragendorff's reagent was prepared as follows:

Solution A: Bismuth subnitrate (850g), H_2O (40 ml) and AcOH (10ml).
Solution B: KI (8g), H_2O (20 ml).

Stock solution: Solution A and B were mixed and strored in a dark bottle.

Spry solution: stock (10 ml), AcOH (20 ml) and H_2O (100 ml), stable for several weeks.

RESULTS

Properties of Fractions of Aristolochia Seeds:

Paper chromatographic results of the alcohol extract and fractions using the solvent system (B.AC.W) and (B.A.W) are given in Table (1).

The alcohol extract gave sports. Two yellow sports were seen in both the ordinary and long wave ultra-violet (U.V.) light, two spots fluorescing yellow and one spot fluorescing blue in the same U.V. light. The blue fluorescing spot was stained heavily orange following spraying with Dragendorff's reagent.

This result indicated the presence of an alkaloid. Evaporation of the ether gave a yellow acidic residue which was found, by paper chromatography, to contain two non-alkaloidal compounds. Three alkaloids (A, B and C) were detected in the quaternary alkaloid chloride. Due to the small amount of the material used, detection of the alkaloid was achieved by streaking a methanolic solution of this fraction on Whattman filter paper No. 17 which was then chromatographed and separated zones were obtained:

A/ Blue fluorescing zone stained with Dragendorff's reagent.

B/ Yellow zone stained with Drragendorff's spray reagent.

C/ Yellow zone stained with Dragendorff's reagent.

The results indicated the presence of three alkaloids in the quaternary alkaloid fraction. Evaporation of petroleum ether resulted in a greenish brown oily residue and was found to contain no alkaloids.

Table 1: Paper Chromatography of Fractions of Aristolochia seeds:

Fraction	No. of sports	Colour in ordinary light	Fluorescence in U.V. light	Rf value	
				B. Ac. W.	B.A.W.
Alcohol	5	-	Blue	0.71	0.03
		Yellow	Yellow	0.91	0.08
		-	Yellow	0.50	0.21
		Yellow	Yellow	0.53	0.39
		-	Yellow	0.78	0.54
Ether	2	Yellow	Yellow	0.91	0.08
		-	Yellow	0.78	0.54
Methylene chloride	1	-	Blue	0.71	0.03
Quaternary	3	-	Blue	0.71	0.03
		Yellow	Yellow	0.53	0.21
		Yellow	Yellow	0.50	0.39

DISCUSSION

It has been suggested that the roots of *A. bracteata* contain Aristolochic acid and aristolochine and probably other compounds (Watt and Breyer-Brandwijk, 1962).

Paper chromatography procedure for the determination of alkaloids in the aqueous extract of the seeds of *A. bracteata* has been developed. Neither the petroleum ether nor the ether extract contained alkaloids. However, The latter contained two non-alkaloidal compound. It is evident that the three alkaloids designated A, B and C were found in the quaternary alkaloid fraction of the seeds of Sudanese species of S. bracteata.

It has been shown that plants of the genus Aristolochia contain different toxic extracts. Lee *et al.* (2002) reported that the major component in *Aristolochia fangchi* was Aristolochic acid I, and the level ranged from 437 to 668 ppm. Aristolochic acid II was the major component of *Aristolochia contorta* and its range was <115ppm.

Rastrelli *et al.* (1997) found that five new protopine type alkaloids, and a novel 8-benzylberberine-type alkaloid, were isolated from the aerial parts of *Aristolochia constricta.*

Three new compounds, Aristolochic acid 111a-6-0-beta-D-glucosidal, cepharanone-A N-beta-D-glucoside and 2-hydrozy-8-methyloxy cepharanone-A were isolated togther with eight known compounds from methanolic extracts of fresh roots of *Aristolochia cinnabarina* (Li *et al.*, 1994).

The aqueous extract of the seeds of the plant has toxic effects in laboratory animals. Barakat, and Fadalla, 11th Sci. Cong. 2004, Fac. Vet. Med. Assiut. Univ., Egypt.

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