

Phytochemical Studies on *Kochia indica* Wight

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In the present study, chemical investigation of the active constituents in the different organs of *Kochia indica* namely, shoot, root, pericarp and seeds was carried out aiming at using them as local raw material for the production of drugs of pharmaceutical value.

The preliminary chemical investigation revealed the presence of carbohydrates and/or glycosides, sterols, tannins, saponins, alkaloids and flavonoids in all studied organs except roots which were free from flavonoids.

The percentage of moisture, ash, water-soluble ash, acid-insoluble ash, soluble-sugars, total carbohydrates, total nitrogen, crude protein, total lipids and crude fibres were estimated. Chromatographic investigations of free and combined sugars, protein-amino acids, organic acids and alkaloids have also been done.

The flavonoids content in the shoots, pericarps and seeds of *Kochia indica* were also studied chromatographically and 4 spots were detected in all organs. One of them was identical to rutin. The physical and chemical properties of the isolated rutin were studied and its amount was found to be 68,127 and 200mg% in shoots, pericarps and seeds, respectively.

The genus *Kochia* has attracted the attention of many authors towards the investigation of its chemical constituents and the possibility of using them in medicinal application. The most important compounds known to be present in the different species of this genus are alkaloids, triterpenes, glycosides, saponins, flavonoids, tannins, sterols (Borkowski and Drost 1965; Tandon and Agarwal 1966; Suh and Shin 1969; Shishkina and Tegisbaev, 1970; Coxworth and Salmon 1972; and Kernan *et al.* 1973).

The present work is an attempt towards chemical investigation of some active constituents in the different organs of *Kochia indica*, namely, shoots, roots, pericarps and

seeds, which were not previously investigated under Egyptian environmental conditions. Of these constituents, carbohydrates, protein-amino acids, organic acids, alkaloids and flavonoids are subjected to separation, identification and estimation.

To the knowledge of the writers nothing is found in the literature about the phytochemistry of *Kochia indica*.

Methods and Results

A – Determination of constants

The phytochemical studies of *Kochia indica* were conducted on the different plant organs, namely roots, shoots, pericarps and seeds. The mean values of moisture, ash, water-soluble ash, acid-insoluble ash, soluble sugars, total carbohydrates, total nitrogen, crude protein, total lipids and crude fibres were determined according to the method of E.P. (1953) and Ward and Johnson (1962).

It is clear from Table 1 that the moisture content was highest in seeds (10.86%), lowest in roots (8.79%) and intermediate in shoots and pericarps (being 9.57 and 10.15%, respectively).

The shoot system contained a relatively high percentage of ash and water-soluble ash being 17.26 and 13.84, respectively while the seeds gave low values reaching 5.70 and 1.50%, respectively.

The root system contained a relatively high percentage of acid-insoluble ash (2.41%), total carbohydrates (6.30%) and crude fibres (37.72%). The seeds contained high concentration of soluble sugars, total nitrogen, proteins and lipids amounting to 1.38, 3.36, 2.10 and 11.40%, respectively.

B – Extraction and screening

The results of extraction with successive selective organic solvents indicated that the seeds gave a relatively high percentage of total residue (84.21%) followed by the pericarps (53.21%), shoots (43.36%) and roots (23.43%). The physical properties of the residues in the different organic solvent fractions of different organs are shown in Table 2.

The preliminary phytochemical screening were carried out following the methods described in E.P. (1953) revealed the presence of alkaloids, carbohydrates and/or glycosides, sterols, tannins, saponins, flavonoids, chlorides and sulphates in all studied plant organs except roots which were free from flavonoids.

C – Investigation of carbohydrates

The free and combined sugars were investigated chromatographically according to Ward and Johnson (1962) methods using ascending one dimensional paper chromatogra-

Table 1. Mean values of the different constants and constituents of *Kochia indica*

Constants and constituents	g %			
	roots	shoots	pericarps	seeds
Moisture content	8.790	9.570	10.150	10.860
Ash content	10.480	17.260	14.420	5.700
Water-soluble ash	4.560	13.840	6.460	1.500
Acid-insoluble ash	2.410	1.240	1.780	0.470
Soluble-sugar content	0.373	0.706	1.300	1.387
Total carbohydrate content	6.295	4.738	1.778	5.167
Total nitrogen content	1.420	1.560	1.560	3.360
Crude protein nitrogen	8.870	9.750	9.750	21.000
Total lipid content	1.190	3.390	3.120	11.400
Crude fibre content	37.720	20.320	20.850	9.800

phy and the running solvent system was n. butanol-acetic acid-water (4: 1: 1%). Aniline phthalate reagent was used to develop the separated sugars spots.

Fig. (1 & 2) show that glucose and sucrose were the free sugars in the roots, shoots, pericarps and seeds of *Kochia indica* while glucose, galactose, fructose, arabinose and rhamnose were the combined sugars present in all studied organs.

D – Investigation of protein-amino acids

The protein-amino acids were extracted and estimated according to Block *et al.* (1958). Sixteen amino acids were detected in all studied organs, namely; lysine, histidine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, tyrosine, methionine, valine, phenyl alanine, isoleucine and leucine (Fig. 3).

E – Investigation of organic acids

The organic acids were extracted with 90% isopropanol at 30–35 °C and subjected to paper chromatography using solvent system of n. butanol-acetic acid-water (4: 1: 5%) for 36hr. After development, the chromatograms were air dried, sprayed with bromophenol blue reagent and the spots were examined in uv light (Rio, 1959). It is clear from Fig. (4) that the different organs of *Kochia indica* contained maleic, oxalic, tartaric, citric and succinic acids.

Table 2. Weight of the residues in the different fractions and other remarks of *K. indica*.

Solvent used	Roots		Shoots		Pericarps		Seeds	
	g %	Remark	g %	Remark	g %	Remark	g %	Remark
Petroleum-ether	1.48	Faint green oily	4.34	dark green resinous mass	15.22	yellow resinous mass	11.86	yellow oily
Ether	1.39	yellow resinous mass	1.55	dark green resinous mass	7.65	yellowish-green resinous mass	5.10	faint yellow resinous mass
Chloroform	0.40	yellow resinous mass	1.57	dark green resinous mass	3.63	yellow resinous mass	3.00	faint green resinous mass
Acetone	0.82	brown resinous mass	0.58	dark green resinous mass	0.96	brown resinous mass	2.75	brown resinous mass
Alcohol	5.88	brown resinous mass	6.01	brown resinous mass	6.97	dark brown resinous mass	20.70	brown resinous mass
Water	13.46	dark brown resinous mass	29.31	dark brown resinous mass	18.78	dark brown resinous mass	40.80	dark brown resinous mass
Total	23.43		43.36		53.21		84.21	

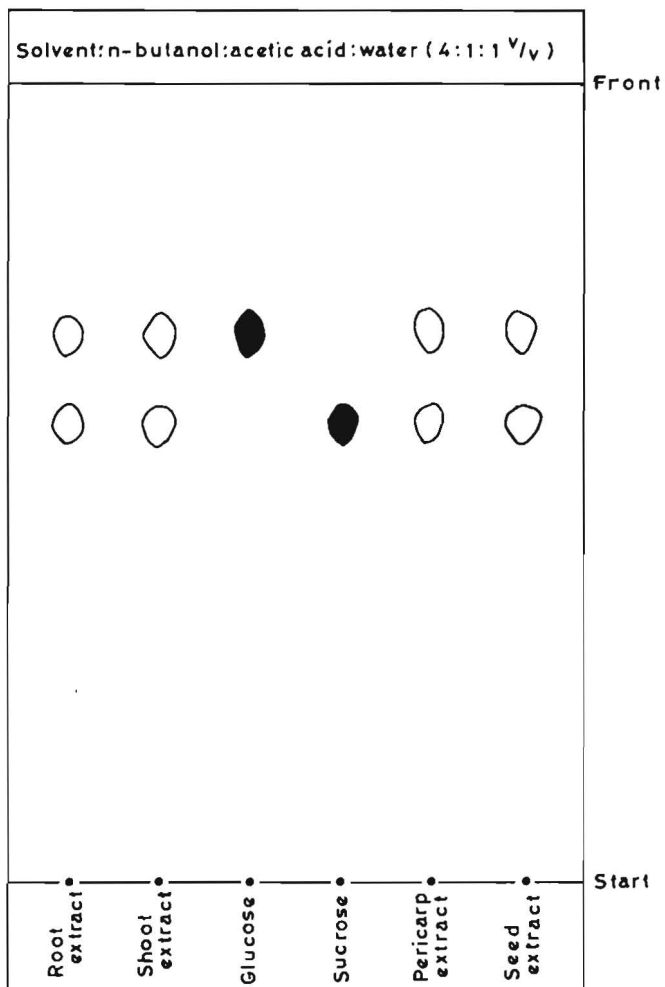


Fig. (1) : Ascending paper chromatogram of the free sugars of *K. indica*.

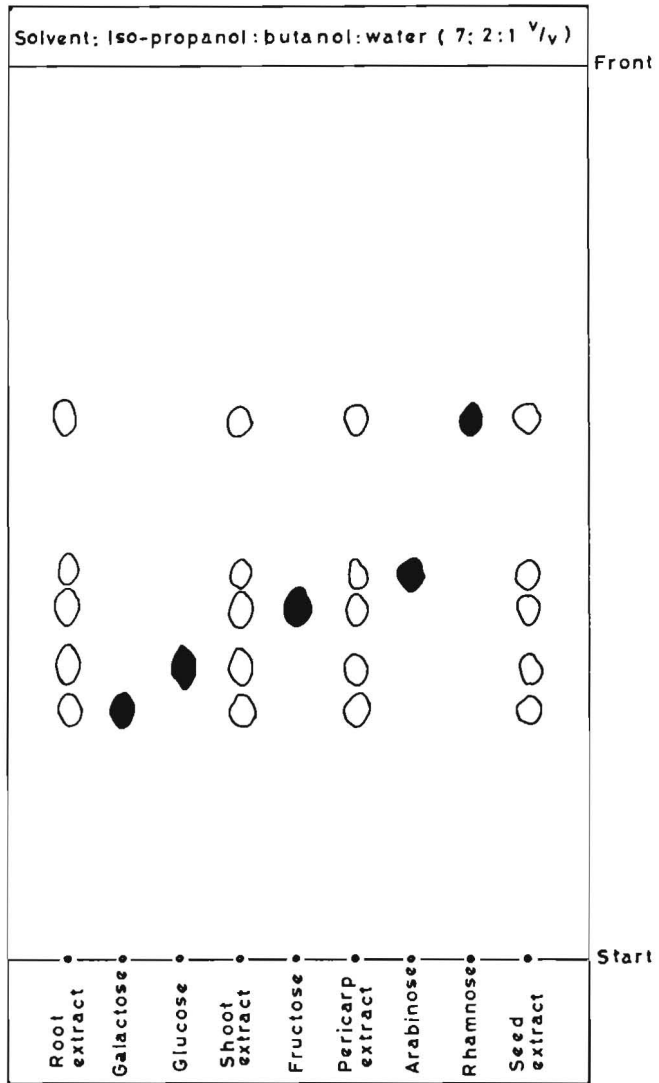


Fig. (2) : Ascending paper chromatogram of the combined sugars of *K. indica*.

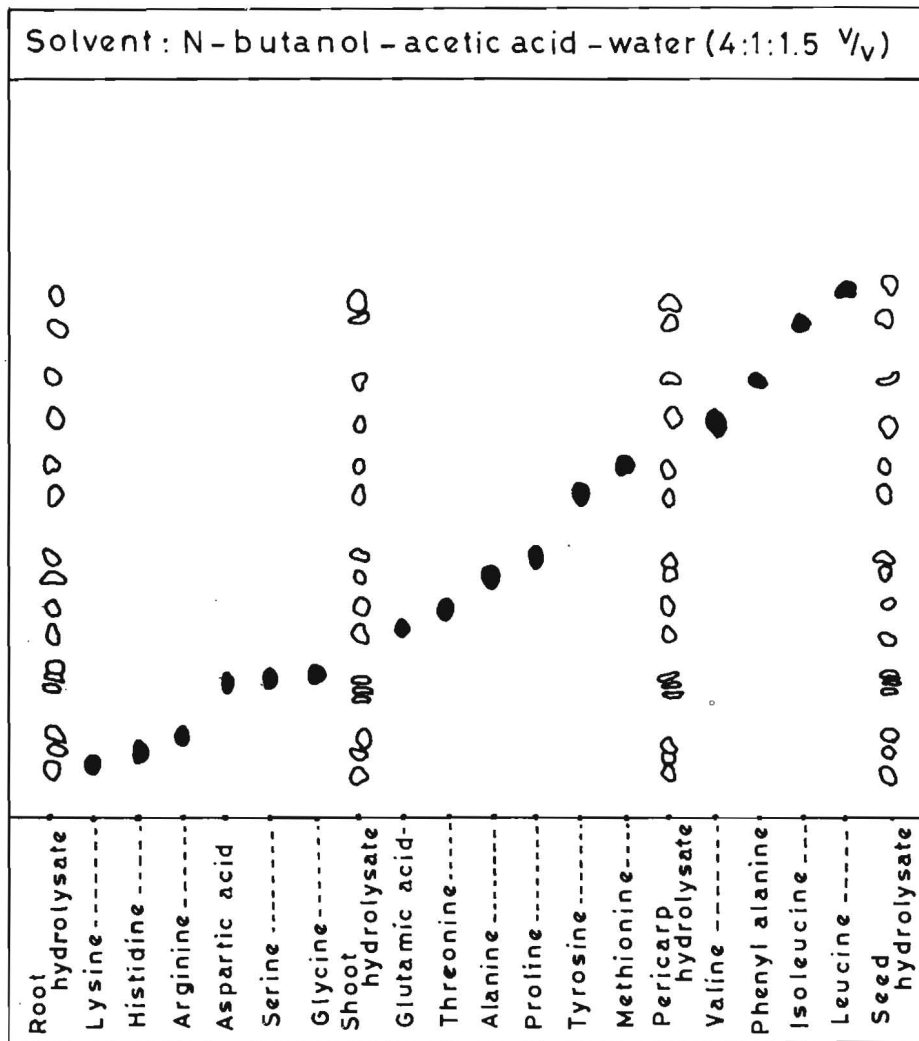


Fig. (3) : Ascending paper chromatogram of the protein amino acids of *Kochia indica*.

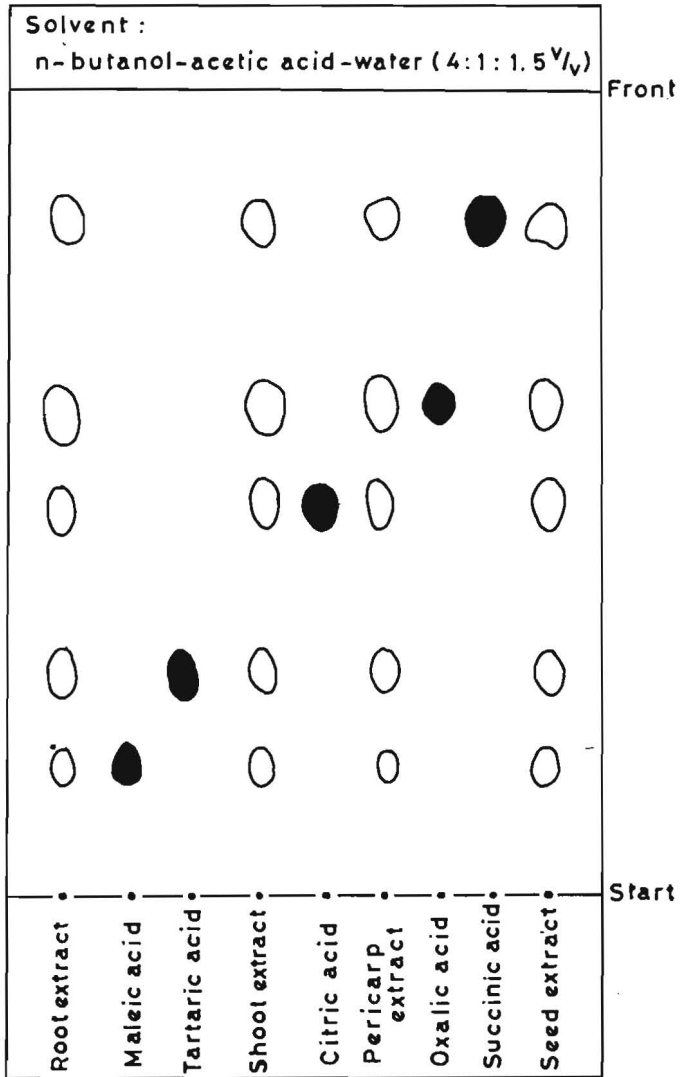


Fig. (4) : Ascending paper chromatographic identification of non-volatile organic acids of *Kochia indica*.

F – Investigation of alkaloids

The alkaloids content in the shoots, roots, pericarps, and seeds of *Kochia indica* were determined as follow: 100g of air-dried powder of each plant organ were extracted with 70% alcohol, concentrated, acidified with 0.1 N H_2SO_4 , filtered and the residue then washed with dilute acid until it became free from alkaloids. The acid solutions were returned alkaline, using NH_4OH solution and the alkaloids were completely extracted with chloroform. The chloroform extract of each studied organ was washed with 5ml distilled water, then evaporated to dryness and the residue dissolved in 40ml of 0.01N sulphuric acid and transferred quantitatively into a measuring flask of 50ml capacity, and completed to the mark with distilled water.

Twenty five ml of each extract was titrated against 0.01N sodium hydroxide solution, using methyl red as indicator.

The data obtained showed that the roots of *Kochia indica* contained a relatively lower value of alkaloids, being 85mg%. The shoot system contained 388mg% and the pericarps contained 387mg%, while the seeds contained the highest value being 512mg%.

The separation of the alkaloids was carried out using thin layer chromatography, covered with silica gel and the running solvent was isopropanol-1/10 N HCl (2: 1%) for 6hr. After development the plates were dried, sprayed with Dragendorff's reagent. It is clear from Fig.(5) that three alkaloids were separated from the roots having R_f values equal to 0.16, 0.35 and 0.46, while shoots, pericarps and seeds contained four alkaloids having R_f values of 0.46, 0.80, 0.83 and 0.85.

Borkowski and Drost (1965) studied the alkaloids found in *Kochia* species. They mentioned that the alkaloids components of *Kochia scoparia* seemed to be very similar to those of *Kochia indica*. The green parts of *Kochia scoparia* contained 3 alkaloids (R_f -values 0.87, 0.88 and 0.91 respectively). Crystals with m.p. of 150–5°C were separated from the last spot. The MeOH-solution fraction when chromatographed, gave one alkaloid (R_f 0.95).

Results of further investigation of the alkaloids of *Kochia indica* will be presented in a forthcoming paper.

G – Investigation of flavonoids

The flavonoidal constituents extracted with 80% alcohol from the defatted material of the shoots, pericarps and seeds of *Kochia indica* were investigated by means of thin-layer chromatographic technique using the solvent system of water-acetic acid – HCl (82: 10: 3%). Four spots were detected in the studied organs after spraying with antimony trichloride. One of these spots (spot No. 3) was identified as the glycoside rutin (Fig. 6).

Ten ml of the concentrated alcoholic extract of the shoots, pericarps and seeds were separately subjected to column chromatography. The column (50cm long and 2cm

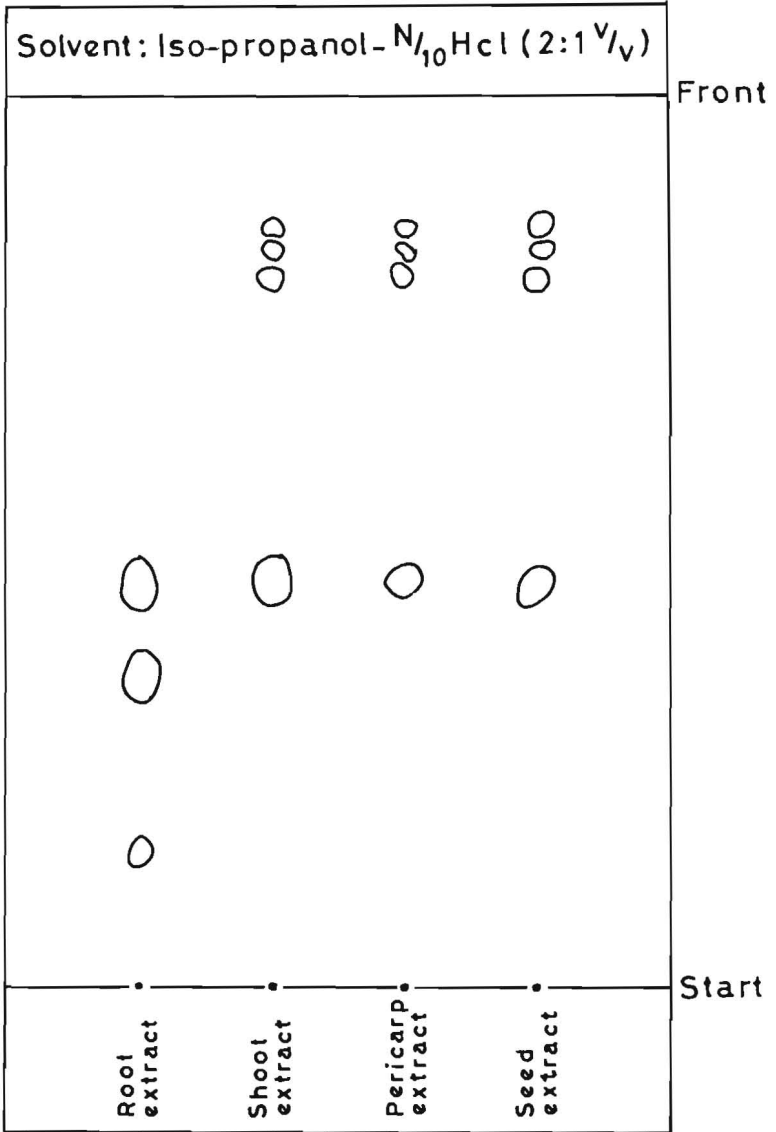


Fig. (5) : Chromatoplate of the alkaloids of *K. indica*.

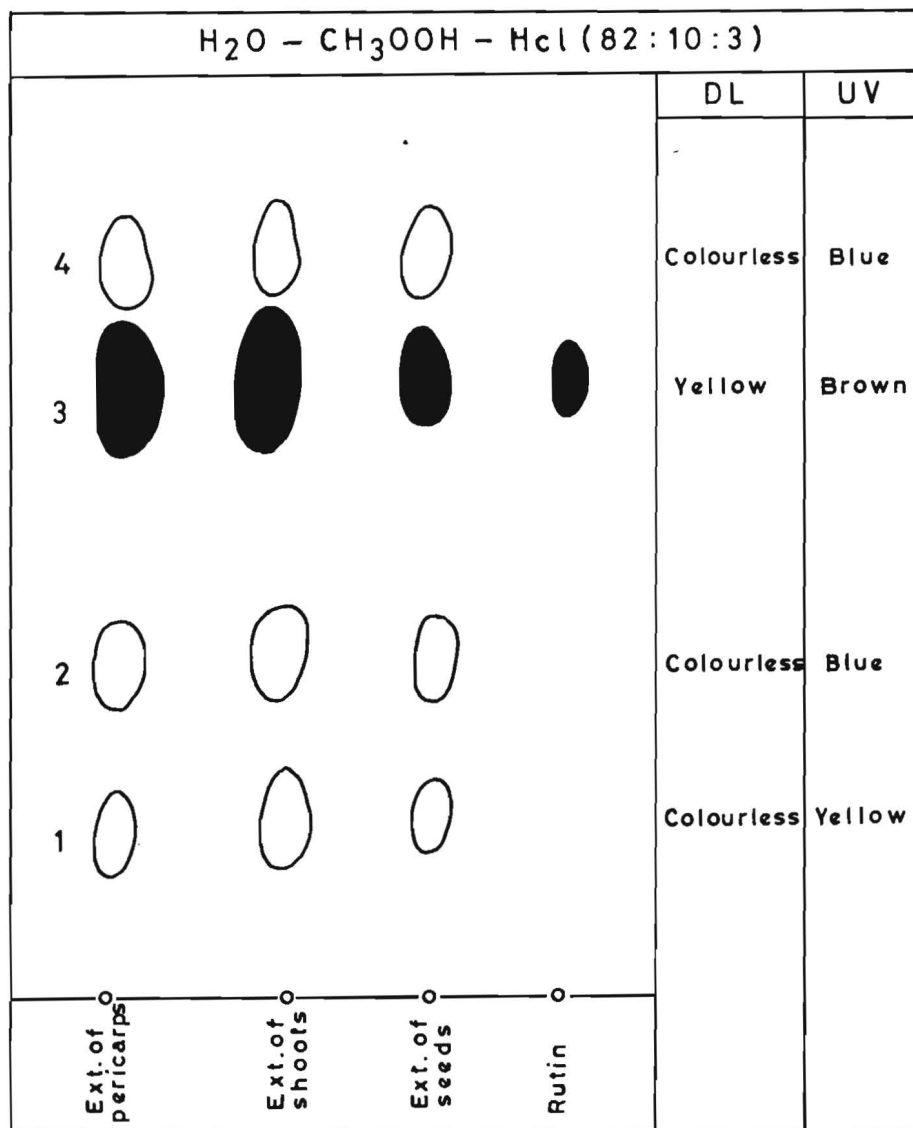


Fig. (6) : Chromatoplate of the flavonoids of *K. indica*.

diameter) was packed with Al_2O_3 to a height of 10cm. The alcoholic extract was gently added to the top of the column where it was adsorbed on the alumina. Elution was made using 100ml of a mixture of petroleum ether: benzene (1: 1%) and the effluent appeared to be greenish in colour. Further elution was made with 80ml of benzene and the effluent was faint yellow in colour. This was followed by the addition of 100ml of equal volumes of benzene and chloroform where a yellow eluate was obtained. With 80ml chloroform, the eluate was yellowish in colour, with 80ml mixture of chloroform: butanol (1: 1%), the eluate appeared faint yellow and with 80ml butanol, the eluate was colourless. At the end of elution, the alumina used become yellow in colour and was then extracted three times with 80% ethanol for 2hr. The combined extracts were concentrated to dryness leaving yellow needle shaped crystals. The separated rutin compound gave the same melting point ($189^\circ C$) and u.v. absorption curve as an authentic one. Also on hydrolysis, it gave glucose and rhamnose as glycone and quercetin as aglycone. A comparison of the infrared spectra of the separated rutin as well as the authentic sample revealed that both were identical (Fig. 7). The amount of rutin determined was 68 mg % for shoots, 127 mg % for pericarps and 200 mg % for seeds.

Conclusions

The phytochemical investigation on the different organs of *Kochia indica* Wight revealed the presence of glycosides, sterols, tannins, saponins, alkaloids and flavonoids except the roots which were free from flavonoids. Of the carbohydrates, glucose and sucrose were detected as soluble sugars; glucose, galactose, fructose, arabinose and rhamnose were detected as the combined sugars. Sixteen amino acids were detected in the hydrolysed protein namely, lysine, histidine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, tyrosine, methionine, valine, phenyl alanine, isoleucine and leucine. The organic acids identified were maleic, oxalic, tartaric, citric and succinic acids in plant. The alkaloid content in roots was relatively low being 85mg% while seeds contained the highest value, being 512mg%. Chromatographic investigation of alkaloids revealed the presence of three alkaloids having R_f values equal to 0.16, 0.35 and 0.46 in roots, while shoots, pericarps and seeds contained four alkaloids having R_f values of 0.46, 0.80, 0.83 and 0.85. The chromatographic investigation of the content of flavonoids in the shoots, pericarps and seeds indicated the presence of 4 spots, one of them was identical to rutin, and its amount in these plant parts was 68, 127 and 200mg% respectively.

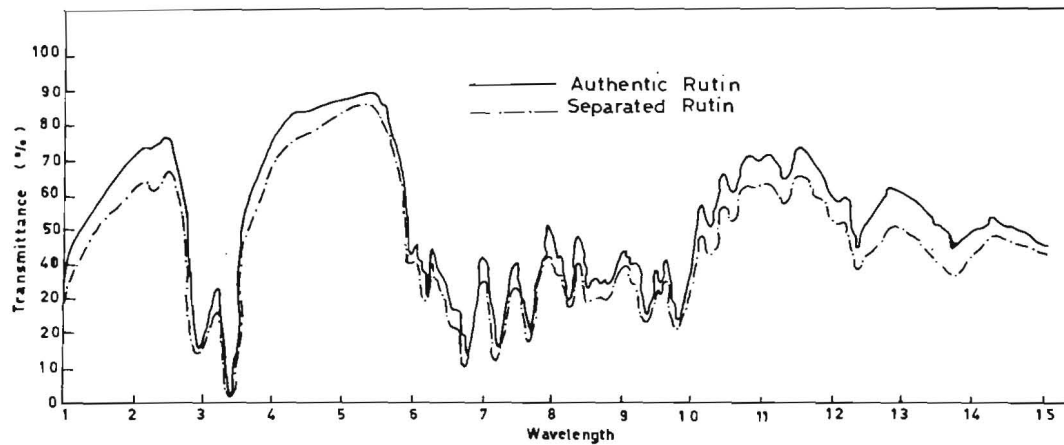


Fig. (7) : Infrared spectrum of the separated and authentic rutin.

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دراسات فيتوكيميائية على نبات الكوخيا انديكا

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يهدف هذا البحث إلى اجراء دراسات كيميائية على نبات الكوخيا انديكا وهو أحد الأنواع النباتية البرية التابعة للفصيلة الرمرامية . وتضمنت هذه الدراسة اجراء فحص كيميائي وتحليل طيفي وكمي للمكونات الموجودة في سيقان وجذور وثمار وبذور هذا النبات ، وقد وجد أنها جميعا تحتوي على سكريات و/أو جليكوسيدات وسترويدات وصابونين وقلويدات وفلافونيدات وراتنجيات وكلوريدات وكبريتات ما عدا الجذور فهي خالية من الفلافونيدات . وقد قدرت الثوابت في أعضاء النبات المذكورة مثل الرطوبة والرماد الذائب وغير الذائب ونسبة السكريات الذائبة والسكريات الكلية ونسبة النيتروجين والهوتين والألياف والدهون . كما تم التعرف على السكريات الطليقة والمعقدة الموجودة بها كفيها بواسطة كروماتوجرافيا الورق وكان الجلوكوز والسكروروز هما السكريات الطليقة أما السكريات المعقدة فبعد تحليلها بحامض الهيدروكلوريك المركز ٦ عياري أعطت جلوكوز وجالكتوز وفركتوز وأرابينوز ورافينوز . كما تم التعرف على الأحماض الأمينية المكونة للبروتين في هذه الأعضاء وكانت ليسين وهستيدين وأرجينين وحمض الاسبارتيك وسيرين وجليسين وحمض الجلوتاميك وثريونين والانين وبرولين واتيروزين ومثيونين وفالين وفنيل ألانين وأيزوليوسين وليوسين . كذلك وجدت خمسة أحماض عضوية وهي ماليك وأكساليك وطرطريك وستريك وسكسنيك ولقد تم تقدير المحتوى القلويدي في كل من السيقان والجذور والثمار والبذور ووجد أن الجذر يحتوي على أقل نسبة (٠,٨٥ %) بينما البذور تحتوي على أعلى نسبة (٥,١٢ %) ويفصل القلويدات بواسطة كروماتوجرافيا الطبقة الرقيقة ووجد أن

الجذور تحتوي على ثلاثة مركبات قلوية بينما السيقان والثمار والبذور فإنها تحتوي على أربعة مركبات .

وقد تم فصل المركبات الفلافونيدية من السيقان والثمار والبذور ووجد أنها تحتوي على أربعة مركبات أحدها تم التعرف عليه وتم فصله في صورة نقية وهو مادة الروتين وكانت نسبته في السيقان ٠,٨٦ ٪, وفي الثمار ١,٢٧ ٪, والبذور ٢٠, ٪.