Chromatographic Identification of Some FlavonoidS Compounds From "CYPERUS ROTUNDAS" Growing In IRAQ

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Abstract:
Alcoholic extract of the tubers of c-rotundas showed the presence at least of three flavonoid compound ( Myricetin , Quercetin and Kaempferol ) by TLC method , using available authentic sample for comparison.

Introduction
The active constituents of Cyprus rotundas(1) which grows in different countries ( China , India , Japan and also in Iraq …etc )
Have been the subject of various studies by several workers(2,3)
The study of flavonoids in the seduges established the presence of the Aureusdin(4) , moreover a wider surry of flavonoids(5) in leaves of sixty-two species from eleven genera of cyperaceae has been undertake by Harborne .The study also revealed that over half the sample contained glycoflavones, one–half tricin a thired luteolin while a sixth contained guercetine and kaemferol .
The results obtained by Harborn have show that Luteolin ,tricin and glycoflavone are characteristic flavonoids in the leaves of the sedage .
The studies of the flavonoide of ninety-two species of Australian(6) Cyperus spp., mainly of tropical and subtropical origin, confirmed a correlation previously reported for this family between flavonoid pattern and plant geography, so the derivatives of the quercetine and kaempferol were reported by Harborne .
The tubers of C-rotundas also were contained to contain(7) glucose, fructose and starch .

Experimental
1. Preparation of plant extract(8,9)
A batch of 100 gm of tubers of C. rotundas was defatted by used a Eoxhlet extractor for 10 – 12 hrs. with 1L per. ether (60 – 80 C°) and the more re – extracted by 1 L ethanol and the extract was concentrated on a vacuum rotatory evaporator at 50 c° to give a dark brown oily (10gm).
2. the preparative(10) Sililca gel (TLC) plates.
The plates were perpetrated with 0.2 mm thickness and heated for 1 hr in a oven at 120 C° then allowed to cool before using.
After compellation of the run in the Jar the plates were dried and the compounds were localized by spraying the plates with aqueous ammonia.
3. NH₃ aqueous(11) as (NH₄OH) solution. It is used for detection of the (falvonoid) compounds.
4. extraction and identification of the flavonoid compounds pretent in ethanolic extract(11)
1 gm of the gum (brown oily) was refluxed with 2N HCL (50 ml) for 1 hr. cooled and extracted with ethyl acetate (2 x 50 ml). the organic layers was dried with anhydrous CaCl₂ and evaporated to dryness.
The residue dissolved in chloroform, was then chromatographed one – dimensionally on Silic gel in two solved systems, BAW (Butanol: Acetic acid: water; 4: 1 : 5, v/v/v ) and forestal (Conc. HCl: HOAc: H₂O; 3: 30: 10, v/v/v).
Both systems gave three spots Rf (0.46, 0.61, 0.84) the former and (0.30, 0.40, 0.74) for the latter, which correspond to Myricetin(1), Quercetin(2) and Kaempferol (3).
Typical Rf – values(12) for flavonoid compounds in two solvents systems are shown in Table (1) and fig (1).

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Rf in BAW</th>
<th>Rf in Forestal</th>
<th>Colour in U.V + Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>0.83</td>
<td>0.55</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.64</td>
<td>0.41</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>0.74</td>
<td>0.53</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.89</td>
<td>0.83</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Chrysoeriol</td>
<td>0.82</td>
<td>0.77</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Myrrcetin</td>
<td>0.43</td>
<td>0.28</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td>Ethyl acetate ext</td>
<td>0.46</td>
<td>0.30</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td>0.40</td>
<td>Bright Yellow</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td>0.54</td>
<td>Bright Yellow</td>
</tr>
</tbody>
</table>
Results and Discussion

Photochemical screening of this plant growing in Qatar, revealed the presence of flavonoids, coumarins and steroids (13).

Moreover, in a previous publication, we have reported the investigation of new phenylpropene compounds from C. rotundus.

In a continuation of the above work, we have noticed the presence of at least three flavonoid compounds in ethanolic extract of the tubers of C. rotundus.

For separation and detection of these compounds we used two solvent systems [BAW: Butanol: Acetic acid: Water, 4:1:5; V/V/V, and Forestall; conc. HCl: HNO₃: H₂O; 3:30:10, V/V/V].

The colours of the spots were visualized by spraying the plates of silica gel with ammonia to give a bright yellow colour. Typical R_f and colours for the most common flavonoid compound are given in Table (1).

A qualitative (TLC) of the ethanolic extract of C. rotundus, and after acid hydrolysis was carried out in silica gel (70 - 230 mesh) using two solvent systems as mentioned before.

Three spots were easily distinguishable, and comparing with quercetin (R_f - value) which only available on authentic sample.

The three flavonoid compounds; Myricetin (1), Quercetin (2) and Kaempferol (3) were visualized as a bright yellow colour under UV lamp after spraying the plates with ammonia; with R_f (0.46, 0.61, 0.84) in the BAW solvent and (0.30, 0.40, 0.74) in the Forestall solvent. Table (1) and (fig 1)

(1)Myricetin, R=OH
(3) Kaempferol, R=H

(2)Quercetin, R=H
Fig (1) chromatogram of some flavonoids as a glycone in ethyl acetate ext. using; BAW and forestall as solvent system
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