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Annals of Biological Research, 2012, 3 (9):4303-4306
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Determination of rutin content in Caper (*Capparis spinosa*) by three analytical methods

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ABSTRACT

Rutin is a bioflavonoid with the strong antioxidant properties which has been found to occur abundantly in some plant .Also it is one of the most abundant flavonoid in Capparis spinosa. The present study was to determine rutin content in all parts of caper plant at the vegetative stage and results of three analytical methods (HPLC and two spectrophotometric) was evaluated.C. spinosa was collected from Tafresh/Iran inJun. Plants separated into leaf, stem and root which were dried separately, and subsequently assayed for rutin content. Among different organs, leaves had higher rutin contents. Comparing three analytical methods, HPLC and $ALCL_3$ are the best method for rutin measurement in C.spinosa.

Key words: *Capparisspinosa*, rutin, HPLC, spectrophotometric methods

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INTRODUCTION

Rutin is one of the bioactive flavonoid compounds which are present in substantial amount in plants. Some related investigations showed that rutin has a broad range of physiological activities[15].Rutin is also an antioxidant[7]and may help prevent the oxidation of vitamin C and have some positive lipid effects[14].Rutin shows anti-inflammatory activity in some animal andin *vitro* models[3][2].Rutinhas a veterinary usein the management of chylothorax in dogs and cats [4].Rutininhibitsplatelet aggregation [10].Rutin's structural formula is presented in fig1.

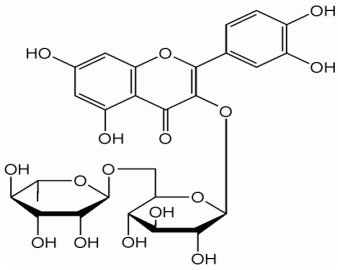

Fig.1: structure of rutin

Rutin is found in many plants, especially the buckwheat plant *Fagopyrum esculentum* Moench, family *Polygonaceae*[5] *Sophora japonica*, *Eucalyptus macrorrhynca*[11]and *capparis spinosa*[8]But only a small number of plant materials contain quantities sufficient for indaustrial extraction[11].

*Capparis spinosa*contains phytosterols,tocopherols, carotenoid, flavonoid and glucosinolates in different parts of plant.The most abundant flavonoid in *C.spinosa* is rutin and the presence of flavonoid rutin makes caper a valuable medicin herb[8]. Around the world numerous Capparis species are collected from their natural habitats to be sold in the herb's seller shops [13].

The objectives of this study were to investigating the variation in rutin content among plant parts and to compare the result obtained by three analytical methods: HPLC and two spectrophotometric methods.

MATERIALS AND METHODS

The caper plants were collected from Tafresh/Iran in Jun at vegetative stage and separated into;leaf,stem and root then were dried at ambient temperature in the shade.

Rutin determination by HPLC:
An amount of 0.1-0.5 g of ground plant material was extracted with 10 ml of solution (methanol-acetic acid- water 100:2:100)for 1 hour on a shaker at laboratory temperature.2ml of the extract were centrifuged for 10 min at 2000 rot/min.Then solution was filtered through a micro filter with a regenerated cellulose membranes of the pore size 0.22 .The filtrate was applied for HPLC.Detection with UV detector was carried out at 355nm. Rutin was eluted at 6.72 min and the peak area was compared to the standard.

The samples used for HPLC were diluted 50 fold. In the$ALCL_3$ method, 0.2ml of 5% $AlCL_3$ in methanol or 0.2 ml of methanol was added to 2 ml of the extract diluted in this way. After 30 minutes, absorbance at 420 nm was measured in solution. Rutin concentration was calculated from a calibration curve as a difference in measured absorbance. For AOAC method (Association of Official Analytical Chemists), the samples used for HPLC was diluted 50 times with a mixture of methanol-aceticacid-water (11:1:8).Absorbance at 352.5 nm and 366.5 nm were measured. For each method calibration graph was drawn and concentration of rutin was calculated according to the published system of equation (fig 2).

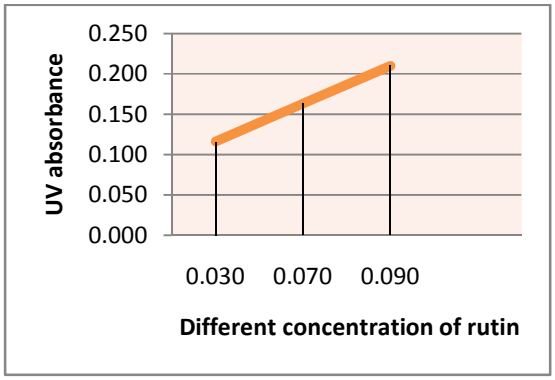

fig.2: Calibration graph for rutin

RESULT AND DISCUSSION

Rutin content significantly was affected by the different parts of plant. Leaves contain the highest rutin content when compare to other organs and followed by stem and root (table 1).

Table 1: Rutin content of different parts of caper

Plant Parts	Rutin content(mg/g)
Leaf	24.72
Stem	4.55
Root	0.38

Comparing the result of three analytical methods, we find that the lowest rutin content were measured by HPLC method. For the leaves $ALCL_3$ method gave on average 25% higher result than HPLC. The method of determining the content of rutin according to AOAC method gave higher result than $ALCL_3$ and HPLC method respectively. The average content of rutin in caper leaves determined by HPLC was 24.72 mg/g dry matter, and it amount to 30.9 mg/g if the spectroscopic method with $ALCL_3$ was used and to 40.32 mg/g dry matter if measured by the AOAC method. The average content of rutin in stem determined by HPLC was 4.55 mg/g dry matter, and it amounted to 5.85 mg/g and 7.68 mg/g with $ALCL_3$ and AOAC method respectively. The results of three analytical methods are given in (figure 2).

Similar to secondary metabolites, tissue-dependence of rutin content is very common among medicinal plants. The comparison between the organs of the *C. spinosa* shows that leaves contain the highest rutin content. Rutin content in the different plant organs and high amount of it in leaves is in good agreement with the result of some other authors.

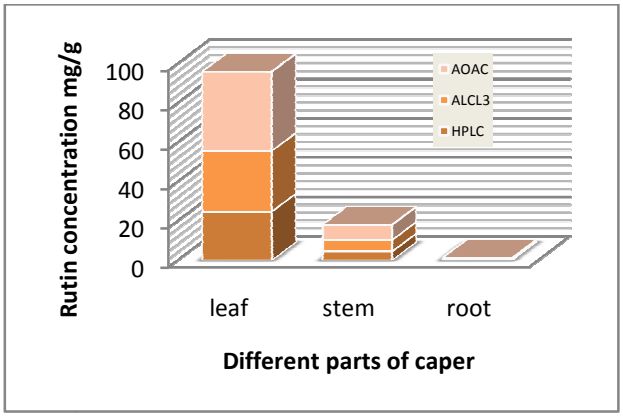

Fig.2 Rutin concentration in the parts of caper.Comparsion the results of three analytical methods

Also high amount of rutin was found in the leaves of caper plant [12][8]. *Hypericum brasiliense* accumulated more rutin in the shoots than other plant parts [1]. Other researcher proved that Rutin content differs in different parts of buckwheat plant (*Fagopyrum esculentum* Moench)in ascending order from hulled grains, unhulled grains, germinated hulled grains, root, germs, stalk, flower, young plants and tops up to leaves[5].

Our finding about three analytical method are in a good agreement with the result of scientists [6][9].They reported higher result for $ALCL_3$ and AOAC than HPLC method. Maybe non-selectivity of $ALCL_3$ method and reaction with other flavonoid lead to higher result than HPLC. It was suggested that no interfering flavonoids that could increase the values measured by the method with $ALCL_3$ were found in Buckwheat samples [9].AOAC method which is optimized to measure rutin concentration in tablet is not suitable way for measurement of rutin in plant material.

COCLUSION

It can be concluded that there is a close relationship between rutin content and caper organ. This study offersleaves of caper as a one source of rutin.

HPLC and $ALCL_3$ method are both suitable method for determination of rutin content in caper.$ALCL_3$ method is not costly and there is no need for any special instrumentation. An AOAC method is not suitable method for determination of rutin content.

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