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DETERMINATION OF TOTAL PHENOLIC AND FLAVONOIDAL CONTENT OF ROOTS OF CYPERUS ROTUNDUS. L

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ABSTRACT

The vast majority of people on this planet still rely on their traditional material medical for their every day health care. The present study was undertaken to investigate the total phenolic and flavonoidal contents, and preliminary phytochemical screening ofroots of cyperus rotundus plant. The total phenolic and flavonoidal contents were measured by Follin-ciocalteau and Aluminum chloride methods respectively. preliminary phytochemical screening techniques were used to test for the presence of some active ingredients in these plant and HPLC analysis total phenolic content. For the plant, the total phenolic content ranged from 62.5 – 48mg Gallic acid/g while total flavonoidal content ranged from 52mg to 30mg Quercetin.

INTRODUCTION

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms and the most studied phytochemical (Fig. 1). 7). Flavonoids can be classified into five major subgroups, these include: flavones, flavonoids, flavanones, flavanols and Anthocyanidins (Nijveldt et al., 2001; Kuhnan, 1976).[4] Flavones are characterized by a planar structure because of a double bond in the central aromatic ring. Quercetin, one of the best described is a member of this group. Quercetin is found in abundance in onions, apples, broccoli and berries. Flavanones are mainly found in citrus fruit, an example is narigin. Flavonoid is involved in scavenging of

oxygen derived free radicals (Nijveldt et al., 2001).[6] It has been identified as a potent hypolipidemic agents in a number of studies (Harnafi and Amrani, 2007 ;Narender et al., 2006).[1,5] It has also been established that flavonoids from medicinal plants possess a high antioxidant potential due to their hydroxyl groups and protect more efficiently against free radical related diseases like arteriosclerosis (Vaya et al., 2003; Kris-Etherton et al., 2002).[3,7] Experimental studies showed that flavonoids enhance vaso-relaxant process (Bernatova et al., 2002)[1] and prevent platelet activity- related thrombosis (Wang et al., 2002).[8]

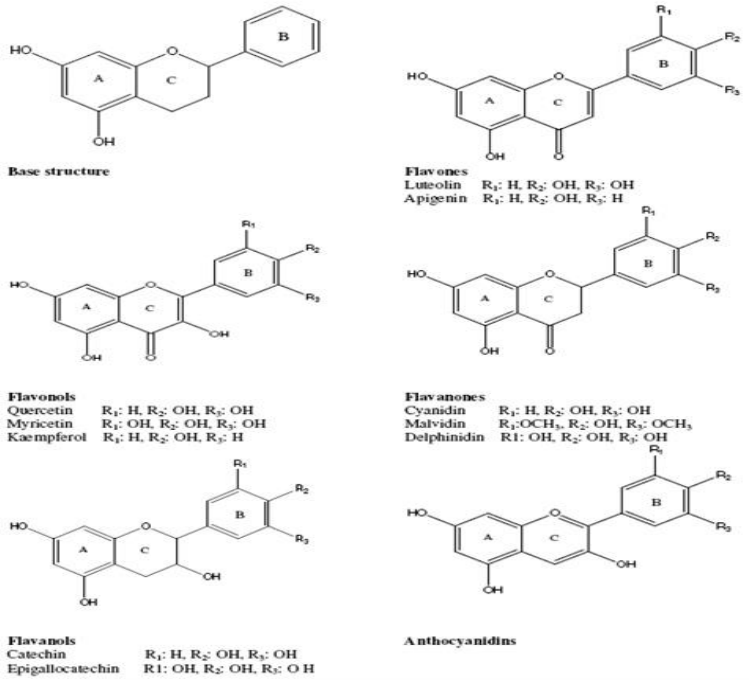

Fig. 1: Chemical Structures of some known flavonoids (Harnafi and Amrani, 2007).

MATERIALS AND METHODS

MATERIALS

Plant materials

Seeds of *cyperus rotundus* L. family *Cyperaceae* were collected from Kosti, White Nile Province, identified by Dr. Hayder Adbalgader and herbarium sheet was deposit at the herbarium of Medicinal and Aromatic Plants Research Institute. (MAPRI).

METHODS

Extraction of plant materials

A. Reagent

- %70ethanol

70ml of A.R ethanol were diluted to 100 ml with distilled water.

B. Procedure

100 grams of coarsely powdered plant material separately extracted by maceration using ethanol (70%) in a conical flask for 72 hours, filtered and the extract was evaporated by a rotary evaporator at 60 oC. The resulting solution was dried at room temperature and kept in a refrigerator until use.

Phytochemical investigation

Test for flavonoids

A. Reagent

- HCl concentrated.
- Magnesium turning.
- KOH:

  1gm of A, R. KOH was dissolved and diluted to 100ml with distilled water.
- Amyle alcohol.
- Methanol.

B. Procedure

Test (1)

To 2 ml ethanol extract, 0.5 ml concentrated Hydrochloric acid and few of magnesium turnings were added in a test tube. Pink-tomato red colour indicates the presence of flavonoids.

Test (2)

To 2 ml ethanol extract, 1 ml of 1% potassium hydroxide solution was added in a test tube. Dark yellow colour indicates the presence of flavonoids.

Test (3)

To 2 ml ethanol extract, 1 ml of 1% aluminium chloride in methanol was added in a test tube. Yellow colour indicates the presence of flavones, flavanones and/or chalcones.

Test (4)

To 2 ml ethanol extract, 0.5 ml concentrated Hydrochloric acid and few drops of amyl alcohol were added in a test tube and shaken. Red colour indicates the presence of flavanoidal glycosides.

HPLC analysis of plant ethanolic extrac

HPLC analysis of plant ethanolic extract was conducted as adopted by Mattila et al. (2000). The method was carried out on HPLC chromatograph (Hewllet Packard®, USA (series 1050); C18 column 250 × 4.6 mm, UV detection at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 35 oC. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. The standards hesperidin, quercitrin, quercetin, naringenin, kaempferol, apigenin and the plant extract (1 µg/ml) were dissolved in 1ml mobile phase and 20 µl were then injected. Retention times and peak areas were used to determine identity and respectively the concentrations.

RESULTS

Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso and Adeyemo, 2007). Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebajoet al., 2009). There is a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes (Eshrat and Hussain, 2002)

Table (1): Phytochemical screening of ethanol extracts of *cyperus rotundus.*

Extract	Alkaloids	Cardiac glycosides	Flavonoids	Tannins	Sponnins	Terpenoids
Ethanoic Extract	++	++	+++	++	+++	+++

+++=highly present, ++ =moderately present

Total flavonoidal content of *cyperus rotundus* plant extract

The total flavonoidal content in the selected plants was expressed as mg Quercetin/g calculated from its calibration curve (Figure)

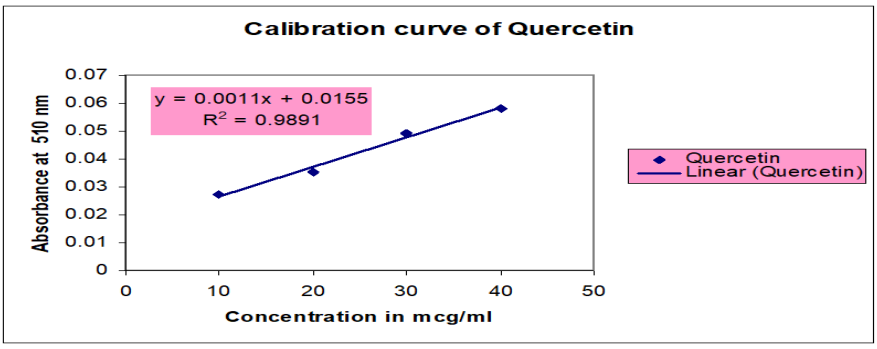

Figure 2: Calibration curve of Quercetin.

Total phenolic content of plant extract

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl group (Hatanoet., al 1989). The total phenolic content was estimated as gallic acid /g calculated from the calibration curve (Figure 3.). the phenolic content varied from 48 to 62.5 mg gallic acid/g of dry materials.

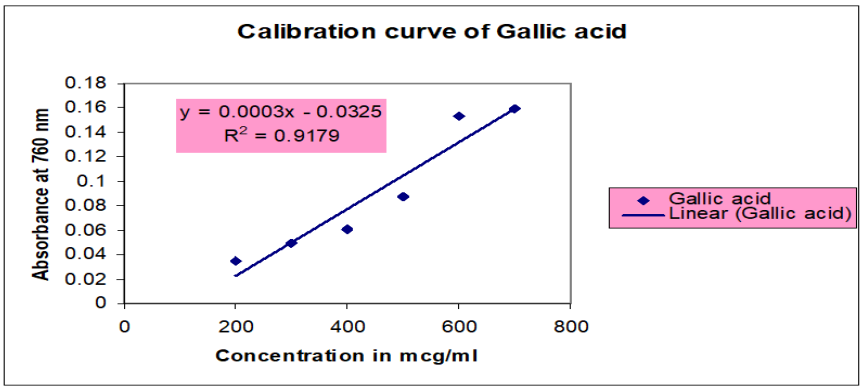

Figure 3: Calibration curve of Gallic acid.

HPLC Analysis of *cyperus rotundus* plant extracts

Identification of flavonoids constituents: different flavonoidal compounds normally have specific chromatographic behavior (retention time t R) and uv spectral characteristic . Identification of the major flavonoids compound in cyperus rotundus plant extract was carried out using HPLC by comparison with flavonoids standard. The flavonoids standards used in this study-were eluted with tR 11.96min to 16.15min in the following order. Hesperidin, Qucetrin, NAringin, Hespertin, Kampterol and Apegnine (fig) shows a typical HPLC chromatogram of flavonoids standars in this study. The major types of flavonoids in the plant include, Hesperidin Qucetrin, Naringin, Kampferol and Apegnine. The cyperus rotundus plant extract had high level of Rotundine A, Rotundine B and Rotundine C aloes contain a series of flavonoids.

HPLC analysis of *cyperus rotundus* plant extracts.

Compound	Test results mg/100g
Catechin	304.99
Qucetrin	180.05
Quercetin	35.67
Myricetin	247.21
Hespertin	-
Kampferol	351.44
Apegnine	107.22

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