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Full Length Research Paper

Antioxidant activity of extracts obtained from residues of nodes leaves stem and root of Egyptian *Jatropha curcas*

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The Egyptian Jatropha crop has been studied for antioxidant activity in ethanol extracts from roots, stem, leaves and nodes. The radical scavenging activity of ethanolic extracts was evaluated with the stable diphenylpicryl-hydrazyl (DPPH) free radical. The antioxidant capacity of the studied organs have been revealed the roots and stem were the strongest in all tests followed by leaves and nodes were the least. All the extracts showed radical scavenging activity. Roots crude extract showed IC_{50} = 0.521 mg/mL. The presence of phenolic compounds and tannins were screened by Folin-Ciocalteau assay, high performance liquid chromatography (HPLC), Ms/MS^n and x-rays diffraction. The reduction power of ethanolic extracts was assayed too. This study suggested the Egyptian *Jatropha curcas* roots and stem are possible sources of natural radical scavengers.

Key words: *Jatropha curcas*, antioxidant activity, DPPH assay, phenolic compounds, HPLC determination, Ms^n – determination.

INTRODUCTION

Plants constitute an important source of active natural products which differ widly in terms of structures, biological properties and mechanisms of actions. Various photochemical components especially polyphenols (such as flavonoids, phenolic acids, tannins, etc.) are known to be responsible for the free radical scavenging and antioxidant activities of plants.

Phenolic substances possess many biological effects. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Bahman et al., 2007). In generally, phenolic subestances all share the same chemical patterns, one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals (Heinonen et al., 1998; Parejo et al., 2002; Lee et al., 2003; Miliauskas et al., 2004; Atoui et al., 2005; Capecka et al., 2005; Galvez et al., 2005; Melo et al., 2005). In recent years, the extracts of many plants have been screened for their antioxidant activities. Phenolic substances such as flavonols, cinnamic acids, coumarins and caffeic acids or chlorogenic acids believed to have antioxidant properties, which are suggested to play an important role in protecting food, cells and any organ from oxidative degradation (osawa,1999; Tikkanen et al., 1998; Wiseman et al., 2000).

In model systems, antioxidants are able to scavenge free radicals and there-by prevent the free radicals from causing damage. Reports indicated that diets rich in phenolic compounds play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular and neurodegenerative disease (Hertog et al., 1993; Anderson et al., 1995; Mauch et al., 2004; Nestle, 2004; Hang et al., 2004). Isolation and identification of these compounds are of interest because of the benefits they contribute. *Jatropha curcas* is one of the selected plants with medicinal value in Africa. It is a small tree or large shrub which can reach a height of up to 5 m.

Normally 5 roots are found from seedings, one central and four peripheral. The purging nut has 5 - 7 shallow lobed leaves with a length and width of 6 – 15 cm, which are arranged alternately. In medicine, preparations of all parts of the plant, including seeds, leaves and bark, fresh

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or as a decoction are used in traditional medicine and for veterinary purposed (Okigbo et al., 2009). Among these, *J. curcas* organs are known for its antioxidant properties and most of its active components have been identified (Hand book of Energy Crops James, 1983).

It has been established that the antioxidant effects are mainly due to the phenolic compounds of the plant (Bandoniene et al., 2002; Miura et al., 2002; Tepe et al., 2006). However, the concentration of the antioxidant compounds could be altered depending on the cultivation conditions, age of plants, climatic conditions and other factors. The importance of antioxidants in the maintenance of health and in protection from the damage induced by oxidative stress (implicated in the risk of chronic diseases), is coming to the forefront of dietary recommendations. Antioxidants offer an effective way to prevent a variety of lifestyle-related diseases and aging that result from lipids per oxidation and active oxygen (Adam, 1995; Ahmadi and Mirza, 1999). Usually synthetic antioxidants such as Butyl Hydroxyl Anisole (BHA) and Butylhydroxyl Toluene (BHT) are used to decelerate these processes because of the possible toxicities of the synthetic antioxidants, increasing attention has been directed toward natural antioxidants (Baser et al., 1996).

Tannins are one of the oldest materials used for tanning hides and skin which converts skin into impurtrescibe leather. Besides tannins giving the usual phenolic reaction, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins (Bate and Swain, 1962). Vegetable tannins are the most com-mon used as hydrolysable tannins in leather industry. Either used alone or accompanying with other tanning agents, they give excellent firmnens and fullness and better light fastness to leather.

Ethanolic extract retains antioxidants such as gallic acid one of the important compounds that is identified as active compounds to prevent allergen and platelet activating factor induce bronchial abstruction in guinea pigs. Galic acid at 1.70 mg/ml inhibit human collagenase by 50%. The gallic acid showed antioxidant and pro-oxidant activities. It decrase the peroxidation of OX brain phospholipids. It also accelerate DNA damage by a ferric-bleomrycin system (Aruma et al., 1993).

Gallic acid and several of its derivatives are naturally occurring plant phenols with anti-mutagenic and anti-carcinogenic activities were tested for their abilities to inhibit the Ornithine Decarboxylase (ODC) response linked toskin tumor promotion. Gallic acid inhibits the ODC response to TPA to a lesser degree than tannic acid (Gali et al., 1991). Chaubal et al. 2005, added that the antioxidative and antiiminobial properties of gallic acid and aliphatic alcohols exhibited inhibitory and antioxidant properties in all tests, including antifungal and tyrosinase inhibition.

The presence of so-called secondary compounds, which of known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity. Secondary compounds include alkaloids, terpenoides, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several different ways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or modify oveiposition. They may retard or accelerate development or interfere with life cycle of insect in other ways. Hence, the industrial utilization of medicinal and aromatic plants should be undertaken with conservation measures in mind.

Presence of flavonoids has been reported from many plants species like *J. curcas* (Saxena et al., 2005). Quercetin can be isolated and extracted from leaf, stem, fruits and root) of many plant species. Jain et al. (2007) mentioned that quercetin has anti-inflamatory antioxidant and anticancer properties which gives medicinally important compound. Ravindranath et al. (2002) were able to isolate coumarino-lignan from *J. curcas* using column chromatography over silicagel. Biswanath and Venkataiah (2000) extracted coumarino-lignoids from Jatropha plant and has been used for the treatment of cancerous growths.

In this paper we investigate the comparative analysis of antioxidant activity of ethanolic extracts from nodes, leaves, stem and roots of Egyptian *Jatropha Curcas*. The antioxidant properties of ethanolic extracts of organs were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Further more, the phenol content is evaluated and reducing power were determined along with spectrophotometry. High Pressure Liquid Chromatography (HPLC), mass spectrometry (MS/MS^n) and X-rays diffraction were used to analyze the extracted residues from different organs of *J. curcas*.

MATERIALS AND METHODS

Plant material

The fresh plant organs of *J. curcas* were collected from trees aged one year, planted in research farm (2 Faddans area, that is, 4.94 acre) at Abu-Rawash in Egypt on February, 2008. The plant was obtained from Institute for soil and water Researches farm.

Preparation of the ethanolic extract

The plant samples were oven-dried (5 days) at 70°C and finally ground up to mesh size (2 mm), seived and extracted by using ethanol. The 2 g sample was extracted in 200 ml ethanol placed in quick fitted conical flask and shaked over night (24) h using a shaker at medium speed. The extract was filtered and kept in the dark at -4°C until tested.

Free radical scavenging capacity (RSC)

RSC was evaluated by measuring the scavenging activity of examined extract on the 2,2-diphenylhydrazil (DPPH) radical. The DPPH assay was carried as described (Philip, 2004) and (Adam et al., 2008). Various concentrations of the sample were mixed with 2 ml (DPPH) solution (0.394 mμ/l) and filled up with ethanol to final volume 10 ml. After 30 min incubation period at room temperature

in dark, the absorbance of the resulting solution and blank (with same chemicals, except for the sample) were recorded against tert-butylated hydroxyl toluene (BHT) as positive control. Three replicates of samples were recorded. The disappearance of DPPH was measured at 517 nm using (Hach DR-2000 spectrophotometer). The percentage of RSC was calculated in the following way:

$$RSC\ (\%) = 100 \times (A_{blank} - A_{sample})/ A_{blank}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

The IC_{50} value, which represented the concentration of the extracts (tested samples) that caused 50% reduction of the initial DPPH concentration calculated from the non linear regression curve of log concentration the tested extract (µg ml^{-1}) against the mean percentage of the radical scavenging activity (IP %).

Assay for phenolic content

Total content of ethanolic extracts were determined by a method described by Matthaus (2002). In brief, 10 mg of the extract was dissolved in 10 ml of ethanol. A 200 µl aliquot of the resulting solution was added to 1 ml of Folin-ciocalteau (diluted 10 folds), 0.8 ml of 2% Na_2CO_3 was added and the volume made up to 10 ml using water-ethanol (4:6) as the diluting fluid. After 30 min the absorbance was measured at 740 nm using a spectrophotometer. The concentration was calculated using tannic acid as a standard and the results were expressed as tannic acid equivalents per gram extract (20 - 80 µg/ml; $R^2 = 0.90$) equivalent per gram of dry weight.

Reducing power assay

The reducing power of the extracted samples was determined according to the method of (Oyaizu, 1986; Shyamala et al., 2004). Different amounts of extracts (0.05 – 5 µg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%)]. The mixture was increased at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of the solution (2.50 ml) was mixed with distilled water (2 - 5 ml) and $FeCl_3$ (0.5 ml, 0.1% 0 and the absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

HPLC method

Each residue was dissolved in 0.5 ml of the solvent consisted of 2.5% butanol: 12.5 methanol: 2% glacial acetic acid: 10% ammonium acetate: 73% water. The dissolved samples were filtered using 0.2 µm filters. Some available standards were prepared at 1 mg/ml concentrations and also filtered similarly before analysis. 20 µl of each sample and standard was subjected to HPLC analysis by isocratic elution following (Shshida et al., 2001). Mobile phase was 11% acetonitrile (CH_3CN) in water at 0.7 ml/mm flow rate. pH, 2.6 was maintained by adding few drops of phosphoric acid (H_3PO_4) in mobile phase. Reversed phase ODS column (4.6 mm × 15 cm) was used.

Column oven temperature was kept constant at 30°C. HPLC pump was LC-10AT (Shimadzu) controlled by SCL- 10Av (Shimadzu) U.VIS. Detection was made at 280 nm wavelength and 0.02 AUFS. Column was washed with 100% acetonitrile after each run. Phenols were identified and quantified by comparing the peak area obtained on similar retention time of the standard peak area with known concentration. As the retention times maybe the same for many compounds for a particular set of HPLC conditions and accurate quantitation may be compromised by co-elution with other analytes.

The separated compounds are then injected into the mass spectrometer where mass data of individual substances are provided according to their mass-charge ratio (M/Z). These compounds can further be subjected to collision-induced dissociation leading into fragmentations. The information obtained together with their mass spectra is important in the elucidation of the structure of a compound of interest and the pattern of fragmentation may allow isomers to be distinguished (Clifford et al., 2003).

MS^n – analysis

MS – equipment (Finning LCQ Advantage MAX). The detector was interfaced with an LCQ deca XP plus mass spectrometer fitted with an Electro Spray Interface (ESI) source (Thermofinnigan) and operating in zoom scan mode for accurate determination of parent ion m/z and in data –dependent, MS^n mode to obtain fragment ion m/z. The software for the control of the equipment and the acquisition and treatment of data was X-calibur.

The phenolic separation

The interface conditions were negative ionization mode, temperature of the capillary 28.56°C , an ionization voltage 4.52 KV, gas flow rate 19.24 arbitrary units and auxiliary gas flow, 0.49 (arbitrary units). The mass detection was performed in the base peak mode, for m/z between 300 and 200. The number of scan events was set as two, which was necessary to fragment the parent ion into daughter ions to give structural information. Irrespectively the number of scan event for tannins was zero giving the stable compound.

X-rays diffraction

The dry residue of ethanol-extracts were scanned using x-rays diffraction (XRD-IRIGAKU Rint – 2500) using Cu Kγ radiation.

RESULTS AND DISCUSSION

Data present in Table 1 indicate the percentage yield obtained after extracting and evaporating *Jatropha* organs residue extracts with ethanol. The *hydroxyl radical scavengers were comparatively higher in root and leaf extracts. Two grams of solid dry Root, leaf, stem and node residues contains around 96.8, 92.40, 86.66 and 35.56% antioxidant components respectively.

The antioxidant activity of *phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxy-gen quenchers, heavy metal chelators and hydroxyl radical quenchers (Rice-Evans et al., 1995) the ethanolic extract tested showed poly phenol content of ethanol extracts was high in root > leaf and lower in stem > nodes at concentration tested 50 and 100 ppm (Figure 1). We therefore suggest that at proper concentration of crude extract of *Jatropha* roots or leaves and stem may act as free radicals scavenger and may react with free radicals

Table 1. Yield, polyphenol content and IC_{50} of *Jatropha* organs extract.

Organ	Yield (wt%)	Polyphenol content (µg/mg)	*IC_{50}
Root	96.8	200	0.048
Leaf	92.4	27.33	0.063
Stem	68.66	22.35	0.047
nod	35.56	4.72	0.73

* IC_{50} = The concentration of the test sample leading to 50% reduction of the initial DPPH concentration calculated from the non linear regression curve of log concentration of the test extract (µg ml^{-1}) against the mean percentage of the radical scavenging activity (IP%).

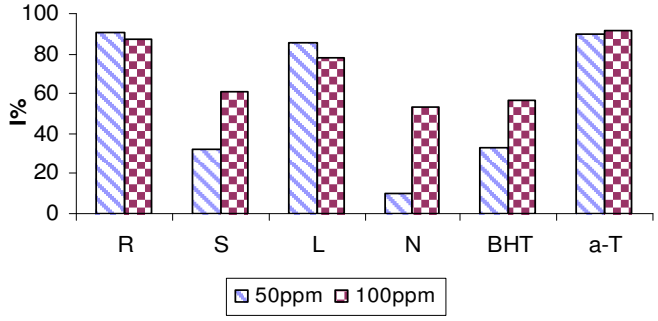

Figure 1. Free radical scavenging activity 1% of Jatropha organs extract as determined by DPPH method.

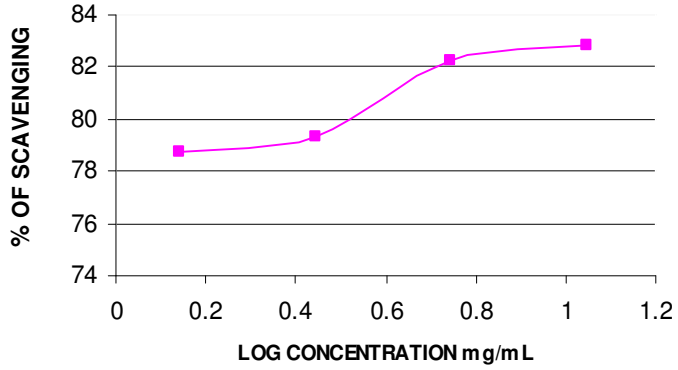

Figure 2. Dose inhibition curve and IC50 values of the crude root extract.

to convert them to more stable products and terminate radical chain reaction (Duh and Yen, 1997). Our results demonstrated that crude extract of *Jatropha* roots has high free radical scavenging activity. Dose inhibition curve and IC_{50} value of crude root extract is shown in Figure 2. In the dose-response experiment it could be observed maximal inhibition was in the rang of 0.521 mg/mL in the presence of 50 mg extract.

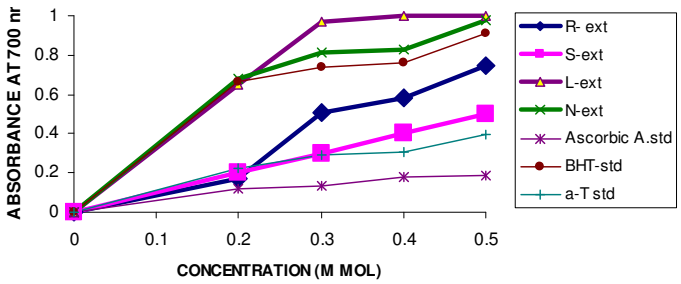

Figure 3. Reducing power for *Jatropha* organs extracts and standards.

Antioxidant effect exponentially increases as a function of the development of the *Reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (Tanaka et al., 1988). Okuda et al. (1983) have reported that the reducing power of tannins from medical plants prevents liver injury by inhibiting formation of lipid peroxides. Reductions are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. As seen in Figure 3 reducing power of ethanol extracts of *Jatropha* organs increased with increasing concentration from 0.1 to 0.6 m mol. Reducing power of organs followed the order – leaves < nodes < root < Stem. The activity of a- trophcorol was lower than the test samples. This is in line with other workers wherein the reducing power of BHT was higher than extracted samples (Chung et al., 2002).

In the present study, the organs extracts of *Jatropha* exhibit a low reducing power they did have an activity that reveals that the root, leaf besides stem and nodes extracts are electron donors can react with free radicals and convert them to stable products thus terminating the radical chain reactions. The reducing power of organs correlated well with the polyphenol content (r_{root} = 200 µg/ml), r_{leaf} = 27.33 µg/ml), r_{stem} = 2.235 µg/ml) and r_{node} = 4.72 µg/ml) (Table 1). Further phytochemical work need to be done on these extracts including fraction to isolate active constituent and subsequent pharmacological evaluation.

HPLC determinations for *Jatropha* residue extracts

The examination of HPLC chromatograms revealed the presence of several compounds. Figures 4 and 5 illustrates the HPLC chromatograms of standard Tannic acids and standard benzoic acid. Results in Figures 6 and 7 indicated the qualitative and quantitative composition of phenols in *Jatropha* organs especially root and stem. Phenols eluted at the same retention times as standard peaks were referred to by the standard phenol name as Gallic acid (R_t = 3.6 min), ellagic acid (R_t = 6.17 min), Quercetin (R_t = 7.11 min), coumaric acid (R_t = 7.19 min), benzoic acid (R_t = 10.3 min) and salicylic acid (R_t = 12.38 min).

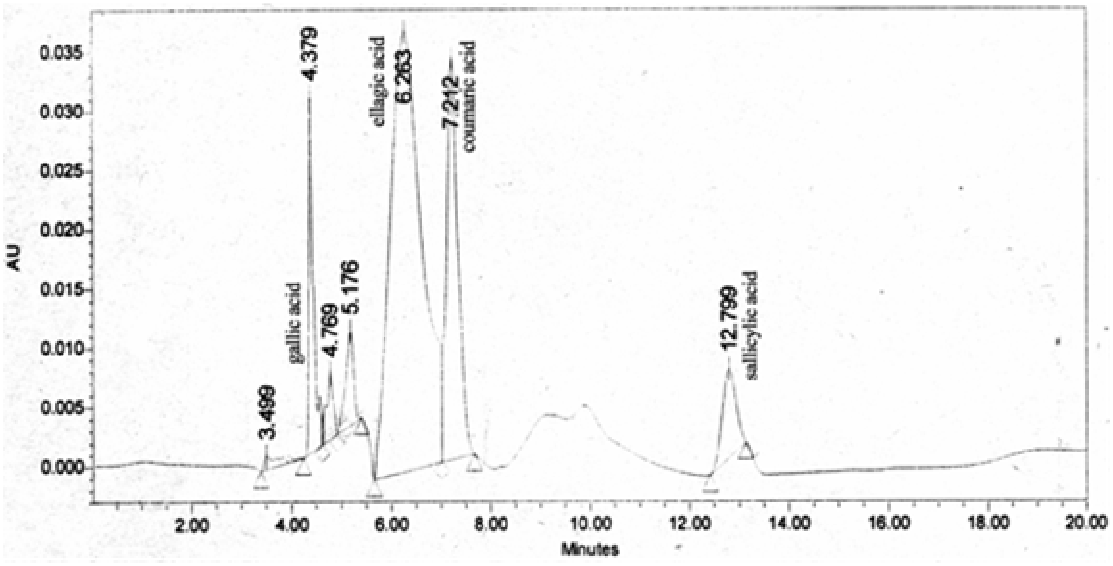

Figure 4. HPLC chromatogram for tannins standards (R_t = 4.769 gallic acid, R_t = 6.263 ellagic acid 60%, R_t = 7.212 coumaric acid 20.36%).

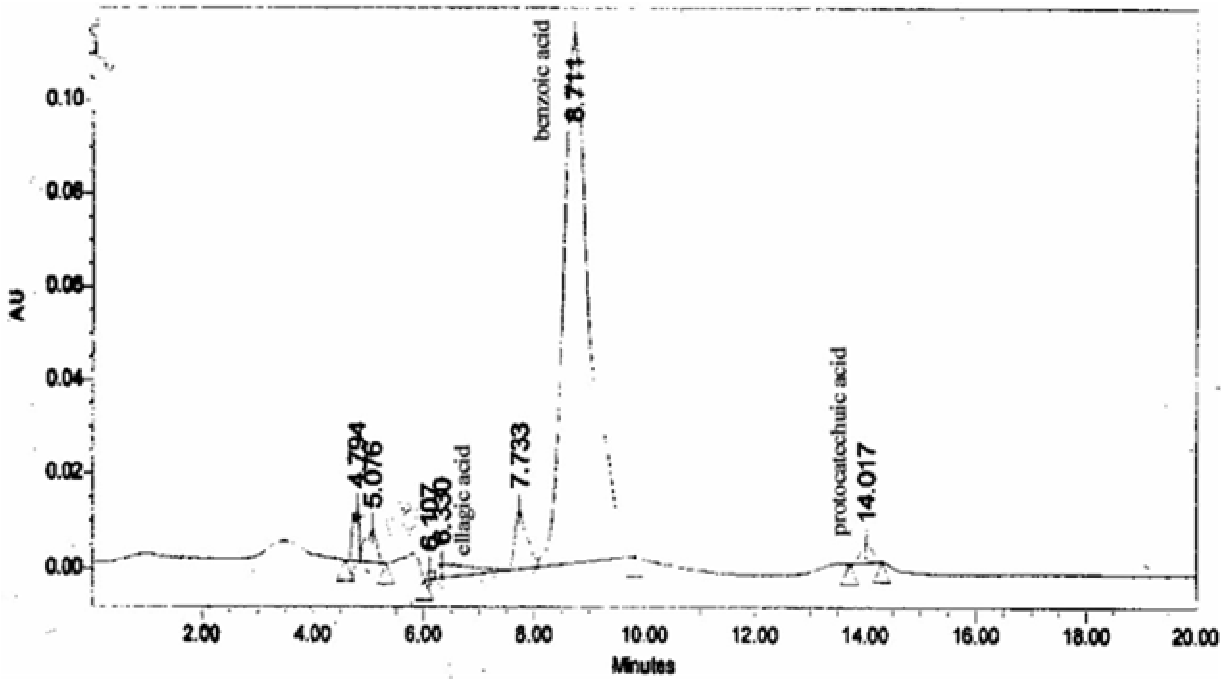

Figure 5. HPLC chromatogram for standard benzoic acid (R_t = 6.33 ellagic acid, R_t = 8.711 benzoic acid, R_t = 14.017 protocatechuic acid).

Gallic acid and benzoic acid were predominant in all investigated samples analyzed using HPLC method. Major quantities differences were seen for both benzoic acid (71.96 and 4.41 mg/ml) and gallic acid being higher in stem (4.39 and 3.41 mg/ml) than its content in root respectively Figures 6 and 7. Coumaric acid possessed 9.18 mg/ml in stem while quercetin possessed 24.65 mg/ml in root. Ellagic acid and salicylic acid were found in traces in stem and root of (3.05 and 5.05 mg/ml) respectively.

MS/MS^n determinations for *Jatropha* residue extracts

The data obtained from this MS/MS^n investigated in

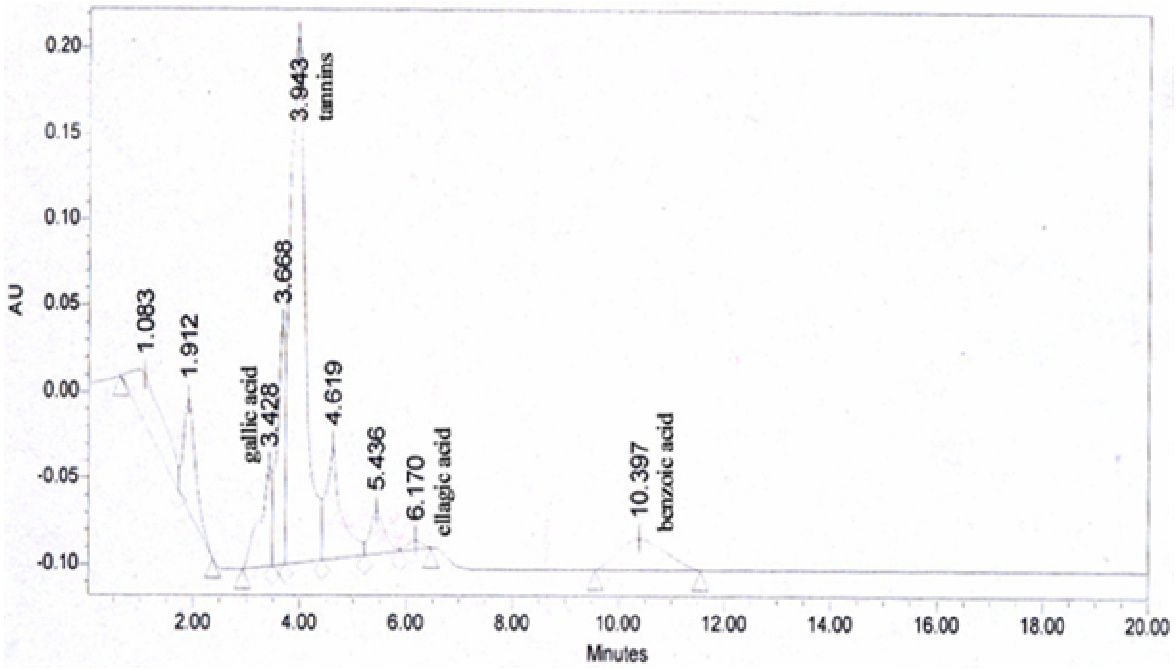

Figure 6. HPLC chromatogram for residue of root extract representing (R_t = 3.428 gallic acid, R_t = 3.943 Tannins, R_t = 6.17 ellagic acid and R_t = 10.397 benzoic acid).

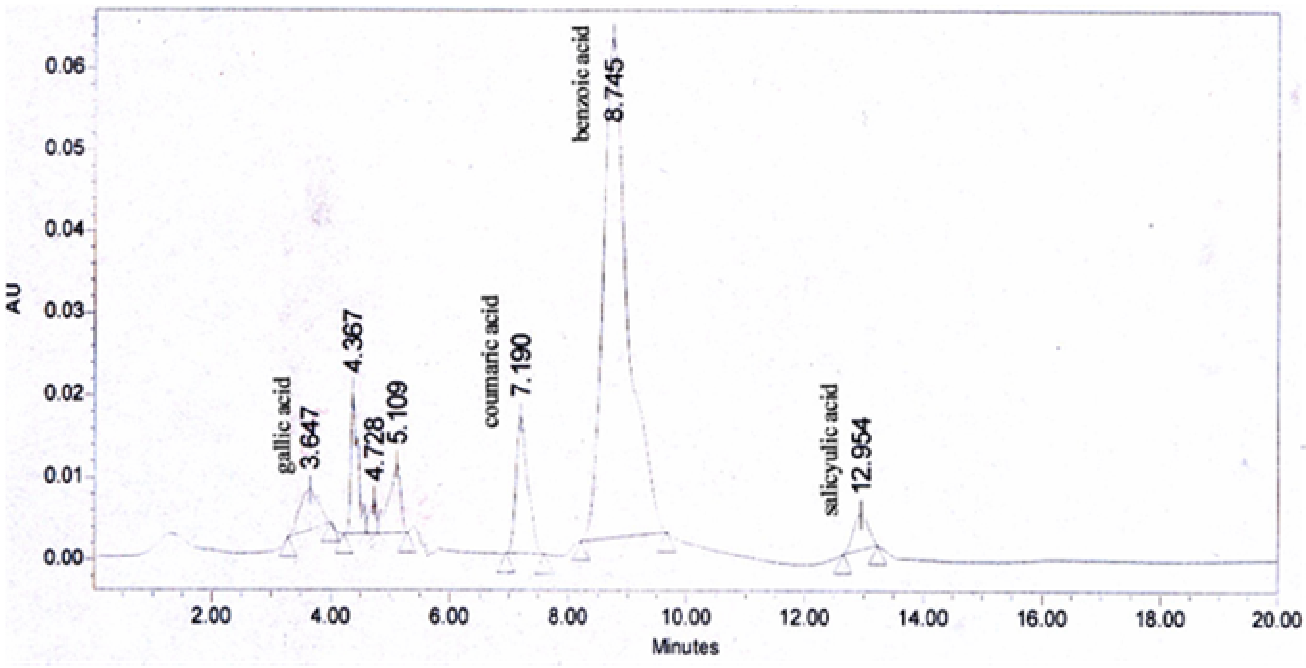

Figure 7. HPLC chromatogram for residue of stem extract representing (R_t = 3.647 gallic acid, R_t = 7.19 coumaric acid and R_t = 8.745 benzoic acid).

Figure 8, strong evidence of the occurrence of hydroxyl benzoic acid and Figure 9 shows presence of gallic acid as major component in ethanolic extracts.

X-Rays determination for *Jatropha* residue extracts

Results in Figure 10 represent x-rays chromatogram

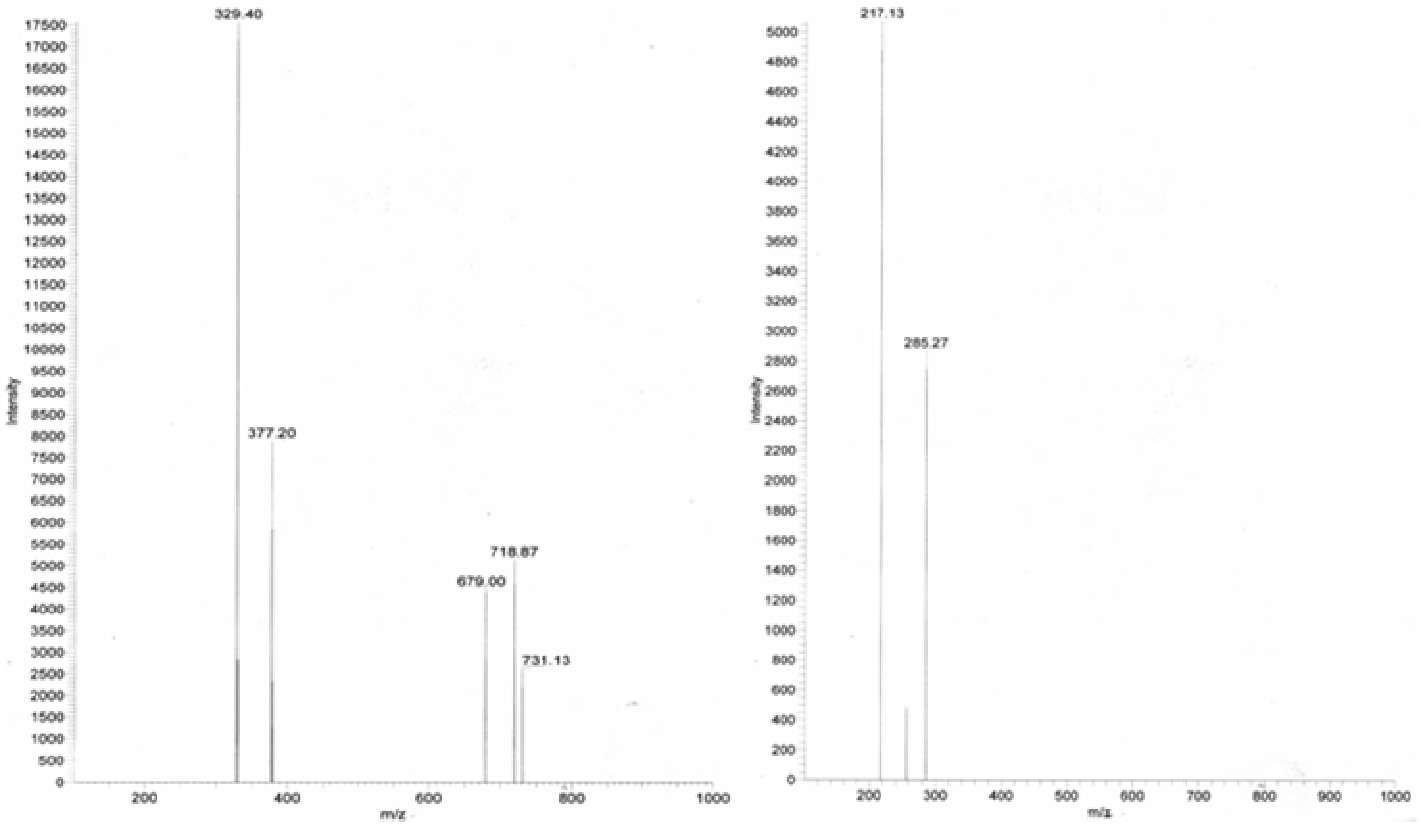

Figure 8. MS/MS^n chromatogram for root extract indicating abundant daughter ions m/z 285 for benzoic acids and its derivatives.

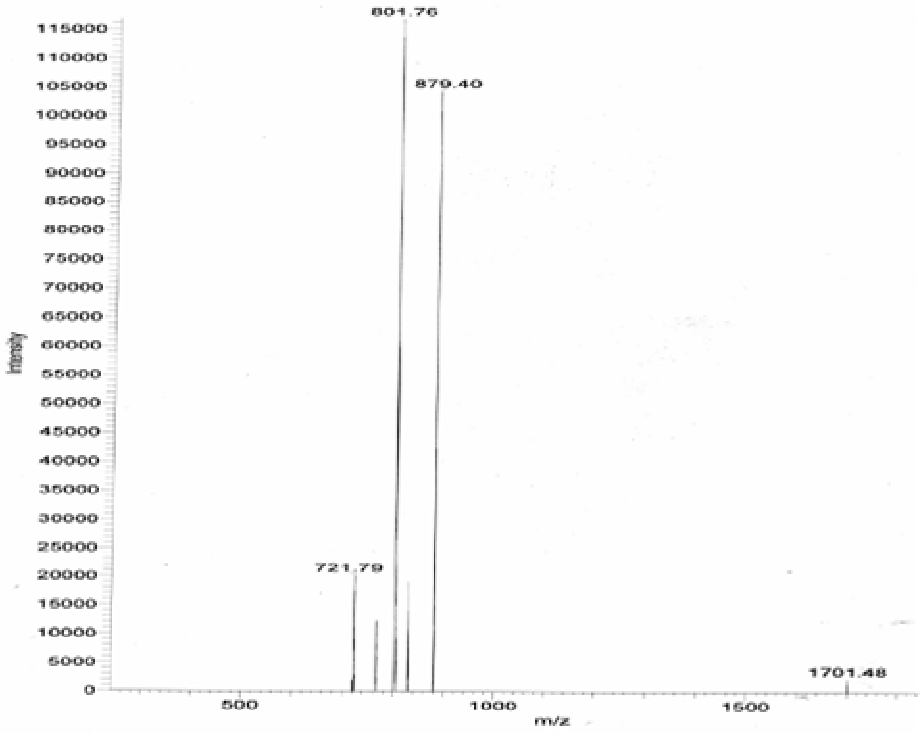

Figure 9. Ms/MS^n chromatogram of stem extract indicating abundant ions m/z 801 and 879 for tannins (gallic acid) as stable constituent.

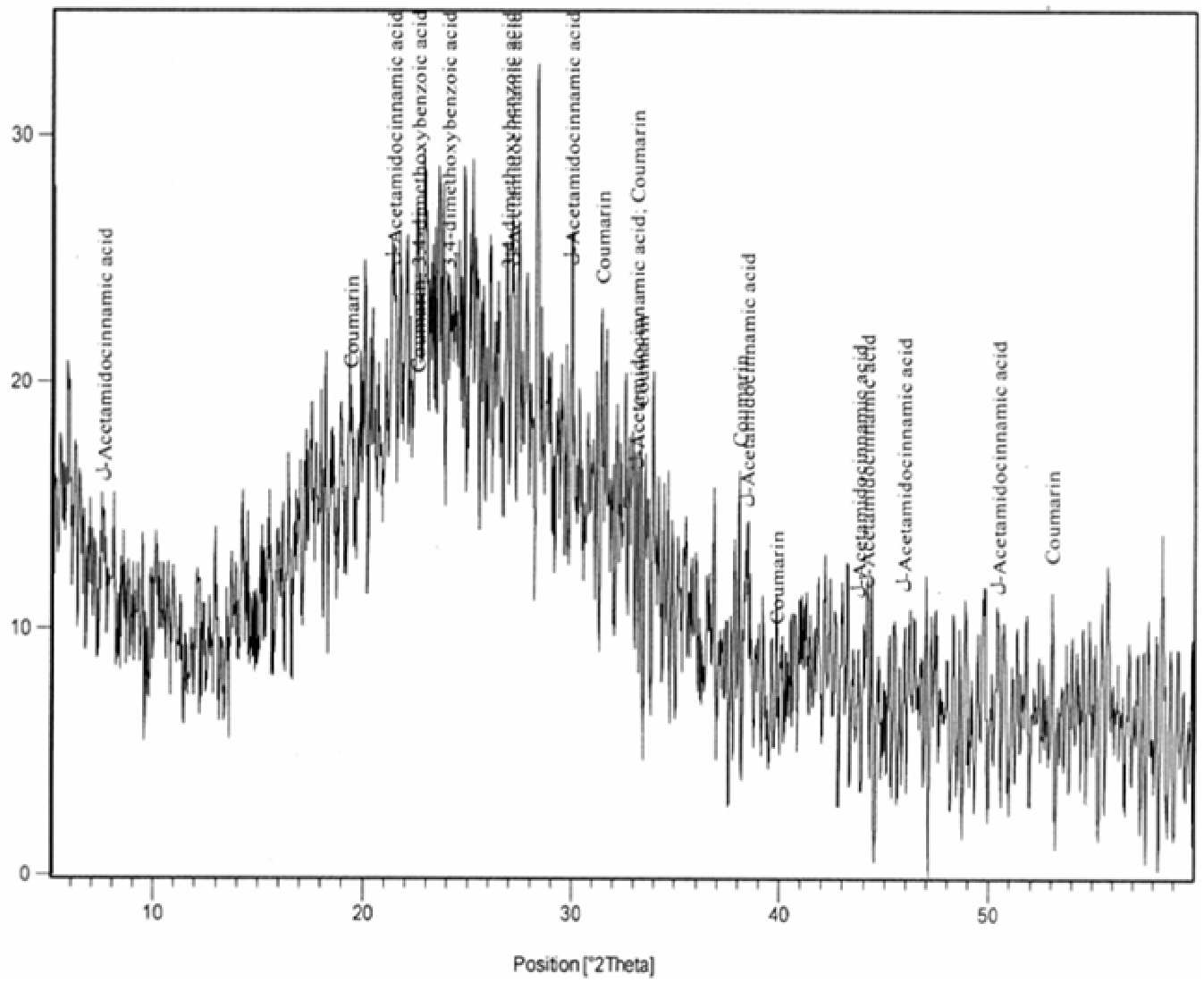

Figure 10. X- rays chromatogram investigating residue root extract showing the presence of benzoic acid, poly(p-phenylenebenzobissoxazole), N-Tosyl-L-glutamic acid.

investigating the occurrence of benzoic acid, poly phenols and traces of propionic and tosylglutamic acid. The chromatogram of residue stem extract in Figure 11 investigates the occurrence of cinnamic acid, coumarin and hydroxyl benzoic acid (gallic acid).

Conclusion

1- The present study indicated that some organs of *J. curcas* (root, leaf, stem and nodes) have different activities. Roots ethanolic extract was the most effective organ in terms of phenolic antioxidant properties followed by leaf and stem.

2- HPLC and MS/MS^n methods have been found to be excellent tools for screening of phenolic compounds in *Jatropha* residues.

3-Hydroxyl radical scavenging activity, reducing ability and poly phenol content of Egyptian *Jatropha* ethanolic extracts were tested by standard procedures indicating root extract an excellent antioxidant and can be substituted for synthetic antioxidants.

4-Therefore, further research is needed for the isolation and identification of other active components in the extracts.

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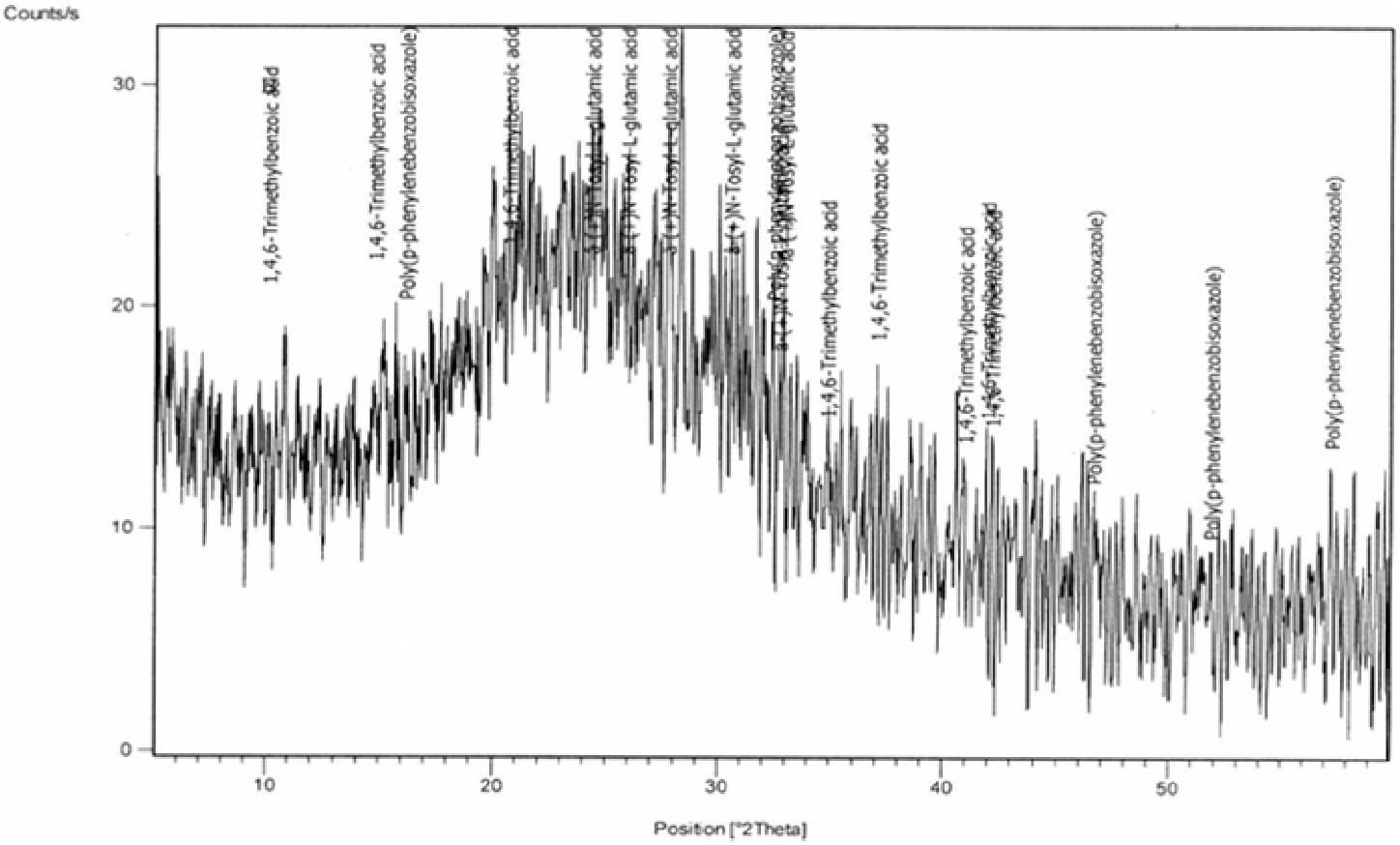

Figure 11. X- rays chromatogram investigating residue stem extract showing the presence of Benzoic acid, cinnamic acid and coumarins.

Research farms from which parts of the *Jatropha* plant were collected.

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