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# EVALUATION OF PHYTOCHEMICALS INTERRELATED TO ANTIOXIDANT POTENTIAL OF UNANI PLANTS

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## ABSTRACT

*Cleome icosandra, Rosa damascena* and *Cyperus scariosus* used extensively in Unani system of medicine to combat various ailments. Extracts of these plants were also noted with antioxidant potential. However, evaluation of bioactive composition of active fractions is essential for its future exploitation as a health-promoting natural product. $IC_{50}$ values of *n*-butanol and chloroform fractions derived from crude extracts and scavenging of DPPH, ABTS, $^{\bullet}OH$, $O_2^{\bullet-}$, $ONOO^-$ and $^{\bullet}NO$, were determined. These active fractions exhibited oxidative DNA damage preventive activity and significant FRAP. No cytotoxicity against U937/PBMC cell lines was noted. Total phenolic and flavonoid content of active fractions was measured. Various phytochemicals viz. quercetin, quercetin-3-o-rutinoside, quercetin-3-o-glucoside, linoleic acid, oleanolic acid, sitosterol, cleomiscosin A and C etc. were identified by ESI-MS analysis of these active fractions and contributing towards the natural antioxidant activity of *C. icosandra, R. damascena* and *C. scariosus*, revealed their potential as a functional food.

**KEYWORDS: Unani plants, Antioxidant potential, Oxidative DNA damage, Phenolics, Flavonoids**

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# INTRODUCTION

In the past few years there has been a growing interest in the reactive oxygen species (ROS) due to their involvement in several pathological situations. When the ROS production is excessive, oxidative damage to proteins, lipids and nucleic acids occurs. These alterations are involved in various pathologies and/or complications[1] such as obesity, atherosclerosis, diabetes, cancer, neurodegenerative diseases, liver cirrhosis and the aging process[2]. To be protected against these toxic effects, the body has developed defensive systems that eliminate free radicals[3]. The polyphenols constitute a large group of chemicals, considered as secondary plant metabolites with different chemical structures (one or more aromatic rings with at least one hydroxy substituent)[4]. Recently, many antioxidants have been isolated from different plant materials [5,6] In this study, we have explored three plants namely *C. icosandra, R. damascena and C. scariosus* regularly prescribed by local practitioners in unani system of medicine to cure various ailments. These plants have been reported several pharmacological activities, e.g. the tubers of *C. scariosus* is credited with astringent, diaphoretic, diuretic etc. *R. damascena* flower buds are astringent and are used in cardiac troubles, etc. Fresh leaf juice from the plant *C. icosandra* has been taken orally for toothache, whereas the seeds have been claimed to have anthelmintic properties[7]. In our previous study, we have shown that the crude extracts of *C. icosandra, R. damascena and C. scariosus* posses' significant antioxidant potential[8]. Hence, the aim of the present investigation is to identify the compounds responsible for its antioxidant activity. For that, sequential fractionations of crude extracts and scavenging assays such as DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-Azinobis-[3-ethyl-benzothiazoline-6-sulfonic acid]), $^{\bullet}NO$ (Nitric oxide), $^{\bullet}OH$ (Hydroxyl radical), $O_2^{\bullet-}$ (Superoxide anion), $ONOO^-$ (Peroxynitrite) and FRAP (Ferric reducing ability of plasma) along with their activity in preventing oxidative DNA damage were done. Cytotoxicity against U937 cells and various photochemical were also identified from *n*-butanol and chloroform fractions which may attribute the antioxidant potential of *C. icosandra, R. damascena and C. scariosus.*

# MATERIALS AND METHODS

### *Plant materials and extraction procedure*

Plants were collected from, and authenticated by, a Unani medical practitioner in Kolkata, India who regularly prescribes these materials. The different plant parts were treated as mentioned previously Kalim et al.[8]. The aqueous layer was lyophilized (at -45 ºC) and the dry powder obtained was partitioned with chloroform (1 L) and water saturated *n*-butanol (0.5 L). The organic layer was further washed with water for complete removal of inorganic impurities, free sugars and other water-soluble residues and then evaporated to dryness under reduced pressure using a rotary evaporator. The fractions were concentrated at room temperature using a rotary vacuum evaporator. *n*-Butanol fractions of *C. icosandra* (3 g), *R. damascena* (4.12 g), *C. scariosus* (3.25 g); and chloroform fractions of *C. scariosus* (4 g), *C. icosandra* (1.72 g) were obtained respectively, which were later used in further investigation.

### *Statistical Analysis*

All assays were performed at least in triplicate. Results are presented as the mean of triplicate runs ± SEM. Statistical analysis was conducted using repeated-measures ANOVA followed by Tukey's test, with statistical significance determined at the $P < 0.05$ or $P < 0.01$ level.

### *Estimation of total phenolics and flavonoids*

The total phenolic content of the extracts was estimated by modified Folin-Ciocalteau method[9] while, the total flavonoid content was estimated by Aluminium chloride method which was described by Zhishen *et al*[10].

### *Reducing power assay*

The ferric reducing power of plant extracts was determined using a modified version of the FRAP assay[11]. A standard curve was

prepared using various concentrations of $FeSO_4 \times 7\ H_2O$.

### *In vitro free radical scavenging assays*

### *DPPH radical scavenging assay*

The radical scavenging activities of the plant fractions were evaluated initially using DPPH, Stock solutions of plant fractions were prepared at a concentration of 10 mg/mL and a freshly-prepared DPPH solution (100 mM) was used as described previously[6].

### *Assay of ABTS radical scavenging activity*

Scavenging of ABTS radical was assayed to assess the antioxidant capacities of the various plant fractions. The ABTS stock solution was prepared by reacting ABTS (7 mM) and potassium persulphate (2.45 mM) and allowing the mixture to stand for at least 16 h to generate ABTS free radicals. The working solution was prepared by diluting the stock solution with methanol such that its absorbance reached 0.7 ± 0.02 at 734 nm ($A_{Control}$)[12]. The % RSC was calculated using the formula

% RSC = $[(A_{Control} - A_{Sample}) / A_{Control}] \times 100\%$.

### *$^{\cdot}OH$ scavenging assay*

The reaction mixture consisted of different concentrations of plant fractions, 3.6 mM deoxyribose, 0.1 mM EDTA, 0.1 mM L-ascorbic acid, 1 mM $H_2O_2$ and 0.1 mM $FeCl_3.6H_2O$, and the volume was made up to 500 µL with 25 mM phosphate buffer, pH 7.4. This mixture was incubated for 1 h at 37 °C, 500 µL of 1% TBA and 500 µL of 1% TCA were added, and the mixture was heated in a boiling water-bath for 15 min and then cooled. The absorbance was measured at 532 nm[6].

### *Non-enzymatic $O_2^{\cdot-}$, scavenging assay*

Superoxide radical was generated *in vitro* by a non-enzymatic method involving the NADH-NBT-PMS system[13]. NBT (150 µM in 0.02 M Tris buffer, pH 8.0) was added to 1 mL of NADH solution (50 µM of NADH in 0.02 M Tris buffer, pH 8.0) in the presence of various concentrations of fractions. The reactions were initiated by adding PMS (15 µM) and the absorbance was at 560 nm was measured exactly 1 min later. Results were recorded as percentage inhibition. Quercetin at various concentrations was used as standard. All tests were performed six times.

### *$ONOO^-$ scavenging assay*

It was measured by a slight modification of Hazra et al.[14]. The reaction mixture consisted of 0.1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, plant fractions at various concentrations and 1 mM peroxynitrite adjusted to a final volume of 1 mL with 50 mM phosphate buffer (pH 7.4). The reaction mixture was incubated at 25 °C for 30 min and the absorbance was measured at 611 nm.

### *$NO^{\cdot}$ scavenging assay: concentration dependence*

The scavenging activity against nitric oxide was assayed (Marcocci et al)[15]. Sodium nitroprusside (0.5 mL, 5 mM in 20 mM phosphate buffer, pH 7.4, previously bubbled with argon) was added to tubes containing 0.5 mL of different plant fractions of various concentrations and incubated at 25 ºC for 150 min. At the end of the incubation, 1 mL of Griess reagent (equal volumes of 2% w/v sulphanilamide in 5% phosphoric acid and 0.2% w/v naphthylethylenediamine dihydrochloride) was added to each sample and the absorbance was measured at 546 nm against control samples and referred to the absorbance of standard solutions of sodium nitrite treated in the same way with Griess reagent. Results were recorded as percentage nitrite formed.

### *Prevention of oxidative DNA damage*

This experiment was performed as described previously[6]. Plasmid pBluescript II SK (-) (250 ng) was treated with $FeSO_4$, $H_2O_2$ and phosphate buffer (pH 7.4) in final concentrations of 0.5 mM, 25 mM and 50 mM, respectively, and test fractions at different concentrations.

### *ESI-MS Analysis of Phytochemicals*

10 mg of *n*-butanol fraction of *C. icosandra* was dissolved in 100 µL methanol and TLC was performed using a mixed solvent of benzene, chloroform and ethyl acetate (6:3:1). Two major spots were visible under UV light (253nm and 365nm). Further preparative TLC was done. For that, samples were loaded in plates, allowed to run in the previous solvent

and the two spots obtained were scratched and dissolved in methanol in two different conical flasks, filtered and were given for MS analysis. 10 mg of *n*-butanol fraction of *R. damascena* was dissolved in 100 µL methanol and TLC was performed using the mixed solvent system of chloroform, methanol and water (7:2.5:0.5). Three spots were visible under UV light (253 nm and 365 nm). Further preparative TLC and MS analysis were performed. 10 mg chloroform fraction of *C. scariosus* was dissolved in 100 µL chloroform and TLC was done using a mixed solvent system of benzene, chloroform and ethyl acetate (6:3:1). Three spots were visible. Further preparative TLC and MS analysis were performed. The compounds so identified were further checked on the TLC plate by running along with the sample reference compounds.

***Cell Viability Assay***

U937- Human leukemic monocytic lymphoma were maintained in RPMI-1640 medium and PBMC were isolated from anticoagulated blood of five healthy donors[16]. The cytotoxic activity of the *n*-butanol fractions of *C. icosandra, R. damascena* and chloroform fraction of *C. scariosus* was evaluated in U937 and PBMC using MTT assay[17]. Briefly, cells (1.25–2.5 X $10^4$ cells/100 µL of RPMI 1640 medium/well) were seeded in 96-well tissue culture plates and incubated with the *n*-butanol fractions of *C. icosandra, R. damascena* and chloroform fraction of *C. scariosus* in 5 and 50µg/mL for 0, 24, 48 and 72 h at 37 °C, 5% $CO_2$. Following treatment, cell viability was measured by adding 20 µL MTT (5 mg/mL in PBS) and incubated for 4 h at 37 °C. Subsequently, 100 µL DMSO was added to each well, resultant optical density were measured at 540 nm in an ELISA Reader (BIO RAD, CA, USA).

## RESULTS AND DISCUSSION

This study demonstrated the antioxidant capacities of active fractions derived from the crude extracts of three Unani plants based on chemical assays which can be correlated with their significant TPC and TFC. The total phenol and flavonoid contents and antioxidant activity of *n*-butanol and chloroform fractions derived from three Unani plants viz. *C. icosandra, R. damascena* and *C. scariosus* was determined. The mean values of phenols ranged from 81.76 to 152.47 mg GAE (Gallic acid equivalent)/g and flavonoids from 74.65 to 164.13 mg QEE (Quercetin equivalent)/g extract. Highest TPC and TFC was observed by *n*-butanol fraction of *C. icosandra* which is *(*152.47 mg GAE/g extract) and (164.13 mg QEE/g extract) as shown in Table 1. Free radical scavenging potential, initially performed by DPPH assay. Out of five fractions, maximum scavenging activity was reported by three plant fractions namely *n*-butanol fraction of *C. icosandra* and *R. damascena* followed by the chloroform fraction of *C. scariosus*, which showed 89.27% inhibition at 14 µg/mL, 85.24% inhibition at 15 µg/mL and 85% inhibition at 20.34 µg/mL concentration respectively. This was followed by an ABTS assay, here three plant fractions had given maximum scavenging activity namely *n*-butanol fractions of *C. icosandra* and *R. damascena* followed by the chloroform fraction of *C. scariosus* which showed 70.% inhibition at 4.23 µg/mL, 9.33 µg/mL and 10.75 µg/mL concentration respectively. To determine this antioxidant potential specifically, fractions were checked for $^{\bullet}OH$ scavenging activity and the highest activity was noted with the *n*-butanol fraction of *C. icosandra* and *R. damascena* followed by the chloroform fraction of *C. scariosus* which showed 78.23% inhibition at 30 µg/mL, 77.93% inhibition at 41.57 µg/mL and 76% inhibition at 43.23 µg/mL concentration respectively, corroborating the previous assay. Significant $^{\bullet}NO$ scavenging potential was also noted with the *n*-butanol fraction of *C. icosandra*, followed sequentially by *n*-butanol fraction of *R. damascena,* chloroform fraction of *C. scariosus*, *n*-butanol fraction of *C. scariosus* and chloroform fraction of *C. icosandra as* shown in Table 2. Peroxynitrite scavenging activities was 72% inhibition at 800.08 µg/mL, 66.24% inhibition at 812.72 µg/mL and 62.72% inhibition at 824.2 µg/mL for *n*-butanol fraction of *C. icosandra*, *n*-butanol fraction of *R. damascena* and chloroform fraction of *C. scariosus* respectively. $IC_{50}$ values of all fractions were shown in Table 2. $O_2^{\bullet-}$ scavenging activity was also significant in the *n*-butanol fractions of *C.*

*icosandra* and *R. damascena*, and chloroform fraction of *C. scariosus*. As is evident from Table 2, $IC_{50}$ values of *n*-butanol fraction of *C. icosandra* (34.49 µg/mL) was highest which was followed by *n*-butanol fraction of *R. damascena* ( 39.56 µg/mL), chloroform fraction of *C. scariosus* (46.25 µg/mL), *n*-butanol fraction of *C. scariosus* (57.5 µg/mL) and chloroform fraction of *C. icosandra* (61.96 µg/mL) respectively. Taken together, these findings indicate that *n*-butanol fraction of *C. icosandra* is a significantly potential candidate as a natural antioxidant with significant free radical scavenging potential followed by *n*-butanol fraction of *R. damascena* and chloroform fraction of *C. scariosus*. The Fenton reaction is a major physiological source of $^{\bullet}OH$, which is produced near DNA molecules in the presence of transition metal ions such as iron and copper[18]. As previous reports suggest, polyphenol-rich diets may decrease the risk of chronic diseases by reducing oxidative stress[19]. We conclude that a significant contributor to DNA damage prevention is the scavenging of $^{\bullet}OH$ by *n*-butanol fraction of *C. icosandra,* (0.32 µg/mL), followed by the *n*-butanol fraction of *R. damascena* (0.42 µg/mL) and chloroform fraction of *C. scariosus* (0.47 µg/mL), respectively, this was corroborated by densitometric analysis as in Fig 1A and Fig 1B. All together eight compounds were identified by ESI-MS positive mode from three active fractions as indicated by their free radical scavenging potential. The quasimolecular ion peak appeared at *m/z* 425.24 $[M+K]^+$ and 485.4 $[M+K]^+$ respectively, (Figure 2A, 2B,) were attributed subsequently for cleomiscosin A and C of *n*-butanol fraction of *C. icosandra* by preparative TLC and identified by ESI-MS. Previous studies have reported that the seeds of *C. icosandra* contain coumarino-lignans such as cleomiscosin A, B, C and D, of which A and C is reported to be antioxidants[20]. The seeds also contain flavones such as viscosin[21]. Three compounds were isolated by preparative TLC and identified from *n*-butanol fraction of *R. damascena*. The quasimolecular ion peak appeared at *m/z* 633.18 $[M+Na]^+$, 487.09 $[M+Na]^+$ and 341.03 $[M+Na]^+$ (Figure 2C, 2D, 2E) were attributed for quercetin-3-o-rutinoside, quercetin -3-o-*β*-D-glucoside and quercetin. Petals of *R. damascena* contain essential oils such as citronellol, geraniol, linalool etc., tocopherol and carotene, flavonoid glycosides such as the glycosides of quercetin and quercitrin, and quercetin and kaempferol[22,23,24]. Whereas three compounds were identified from the chloroform fraction of *C. scariosus* such as oleanolic acid, linoleic acid and *β*-sitosterol, their molecular ion peaks appeared at *m/z* 479.38 $[M+Na]^+$, 301.16 $[M+Na]^+$, and 437.23 $[M+Na]^+$ (Figure 2F, 2G, 2H) respectively. Among these. linoleic acid is a fatty acid which is responsible for antioxidant activity[25]. Oleanolic acid is a natural triterpenoid which has recently attracted considerable attention for its antioxidant properties[26]. Sitosterol is a phytosterol are reported to have antioxidant activity[27]. *n*-Butanol fraction of *C. icosandra, R. damascena* and chloroform fraction of *C. scariosus* were unable to inhibit proliferation on cancer cell line U937, as well as normal cell human PBMC. These results indicate that these three active fractions namely, *n*-butanol fractions of *C. icosandra, R. damascena* and chloroform fraction of *C. scariosus* do not confer any toxicity to both cancer and healthy, normal cell lines as shown in Table 3A and 3B.

## Table 1
### *Total phenolic, flavonoid content and Ferric reducing capacity of different fractions obtained from three plants namely C. icosandra, R. damascena and C. scariosus.*

| Plant Name | TPC (mg GAE/g plant extract) | TFC (mg QEE/g plant extract) | FRAP (mmol $Fe^{2+}$/g) |
|---|---|---|---|
| *C. icosandra* (A) | 81.76 ± 0.42[b] | 74.65 ± 0.49[c] | 0.89±0.09[b] |
| *C. scariosus* (B) | 97.72 ± 0.23[a] | 93.24 ± 0.26[b] | 2.12±0.01[c] |
| *C. scariosus* (A) | 116.37± 0.89[e] | 129.43 ± 0.87[d] | 3.08±0.07[a] |
| *R. damascena* (B) | 138.96 ± 0.52[c] | 157.99 ± 0.54[a] | 4.23±0.06[a] |
| *C. icosandra* (B) | 152.47 ± 0.48[a] | 164.13 ± 0.69[c] | 5.89±0.10[d] |

***All values are expressed as the mean ± SD (n = 3). Values in the same column followed by different letters are significantly different (P < 0.05).***
***(A) Chloroform fraction of respected plant***
***(B) n-Butanol fraction of respected plant***

## Table 2
### *Radical scavenging activity ($IC_{50}$) of active fractions of three plants.*

| Plant Name | $DPPH^{\cdot}$ | $ABTS^{\cdot+}$ | $^{\cdot}OH$ | NO | $O_2^{\cdot-}$ | $ONOO^-$ |
|---|---|---|---|---|---|---|
| *C. icosandra* (A) | 17.7±0.23[c] | 12.65±0.72[a] | 14.65±0.22[d] | 250.47±0.74[a] | 61.96±0.15[c] | 970.91±12.11[d] |
| *C. scariosus*(B) | 13.88±0.52[b] | 9.935±0.45[b] | 12.28±0.46[c] | 235.94±0.58[b] | 57.5±0.59[d] | 813.06 ± 5.99[c] |
| *C. scariosus*(A) | 8.96±0.95[d] | 4.16±0.92[d] | 9.74±0.98[b] | 221.62±0.38[d] | 46.25±0.32[a] | 625.41±9.54[b] |
| *R. damascena*(B) | 8.231±0.056[a] | 3.315±0.55[e] | 8.24±0.77[d] | 209.4±0.69[c] | 39.56±0.85[b] | 590.57± 2.78[a] |
| *C. icosandra* (B) | 7.87±0.82[c] | 2.82±0.64[c] | 7.89±0.65[a] | 172.1±0.85[e] | 34.49±0.67[b] | 546.53±6.23[c] |
| Reference Compound | | | | | | |
| Quercetin | 3.21±0.11[c] | 1.34±0.08[a] | 7.42 ± 0.32[d] | 19.23 ± 0.42[b] | 41.98 ± | — |
| Gallic acid | — | — | — | — | 0.95[c] | 820.12 ± 27.34[a] |

***All values are expressed as the mean ± SEM (n = 3). Values in the same column followed by different letters are significantly different (P < 0.05).***
***(A) Chloroform fraction of respected plant***
***(B) n-Butanol fraction of respected plant***

## Table 3A
### *Cytotoxic activity of three active fractions on U937, at two different concentrations*

| Time | Cytotoxic activity (%) | | | | | |
|---|---|---|---|---|---|---|
| | ***n*-Butanol fraction of *C. icosandra*** | | ***n*-Butanol fraction of *R. damascena*** | | **Chloroform fraction of *C. scariosus*** | |
| | 5 µg/mL | 50 µg/mL | 5 µg/mL | 50 µg/mL | 5µg/ml | 50 µg/mL |
| 0 h | 100 | 100 | 100 | 100 | 100 | 100 |
| 24 h | 99.46 | 97.77 | 99.63 | 98.6 | 99.8 | 98.36 |
| 48 h | 99.28 | 97.49 | 99.06 | 98.07 | 99.29 | 97.49 |
| 72 h | 98.79 | 97.06 | 98.73 | 97.84 | 99.05 | 96.64 |

***Each data point represents the Mean± SEM of at least three independent experiments in triplicate.***

## Table 3B
### *Cytotoxic activity of three active fractions on PBMC, Human Peripheral Blood Mononuclear Cells at two different concentrations*

| Time | Cytotoxic activity (%) | | | | | |
|---|---|---|---|---|---|---|
| | ***n*-Butanol fraction of *C.icosandra*** | | ***n*-Butanol fraction of *R. damascena*** | | **Chloroform fraction of *C. scariosus*** | |
| | 5 µg/mL | 50 µg/mL | 5 µg/mL | 50 µg/mL | 5µg/mL | 50 µg/mL |
| 0 h | 100 | 100 | 100 | 100 | 100 | 100 |
| 24 h | 99.89 | 99.19 | 99.1 | 98.09 | 99.72 | 98.65 |
| 48 h | 99.81 | 98.78 | 98.6 | 97.86 | 99.49 | 98.44 |
| 72 h | 99.76 | 98.62 | 98.55 | 97.54 | 99.38 | 98.09 |

***Each data point represents the Mean± SEM of at least three independent experiments in triplicate.***

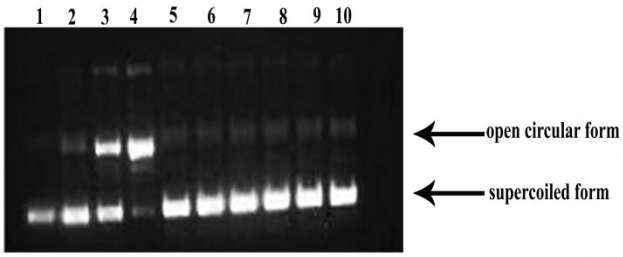


**Figure 1A**

*Electrophoretic pattern of pBluescript II SK (–) DNA breaks by ˙OH generated from the Fenton reaction and prevented by different plant fractions. Lane 1: untreated control DNA (250 ng), lane 2: ~~only~~ $H_2O_2$ (25 mM) + DNA (250 ng) lane 3: ~~only~~ $FeSO_4$ (0.5 mM) + DNA (250 ng) lane 4: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng), lanes 5: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) + quercetin (1mM), lane 6: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) + n-butanol fraction of C. icosandra (0.32 µg/ml), lane 7: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) n-butanol fraction of R. damascena (0.42 µg/ml), lane 8: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) + n-butanol fraction of C. scariosus (0.55 µg/ml), lane 9: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) + chloroform fraction of C. scariosus (0.47µg/ml) and lane 10: $FeSO_4$ (0.5 mM) + $H_2O_2$ (25 mM) + DNA (250 ng) + chloroform fraction of C. icosandra (0.63 µg/ml) respectively (n = 3).*

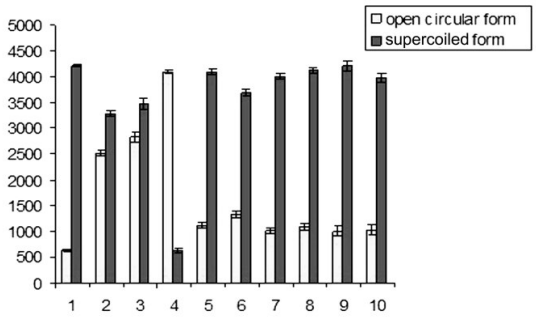


**Figure 1B**

*Densitometric analysis of open circular and supercoiled DNA damage induced by •OH generated from the Fenton reaction in the presence or absence of active fractions of three plants (mean ± SD, n = 3 ).*

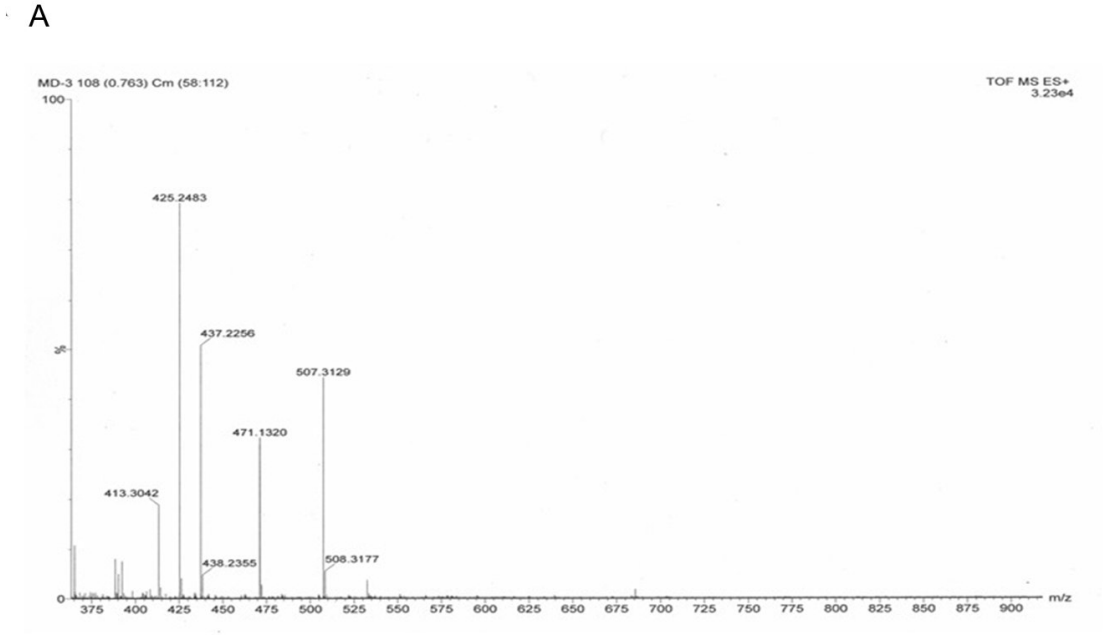


**Figure 2A**

***ESI-MS spectra of compound Cleomiscosin A in C. icosandra.***

B

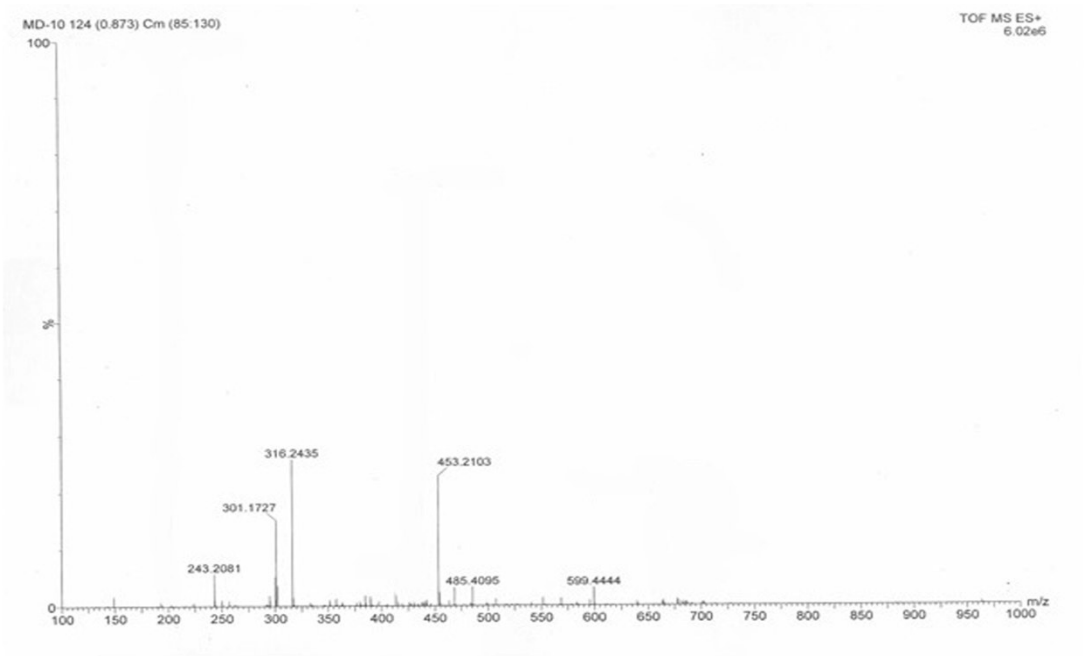


**Figure 2B**
***ESI-MS spectra of compound Cleomiscosin C in C. icosandra.***

C

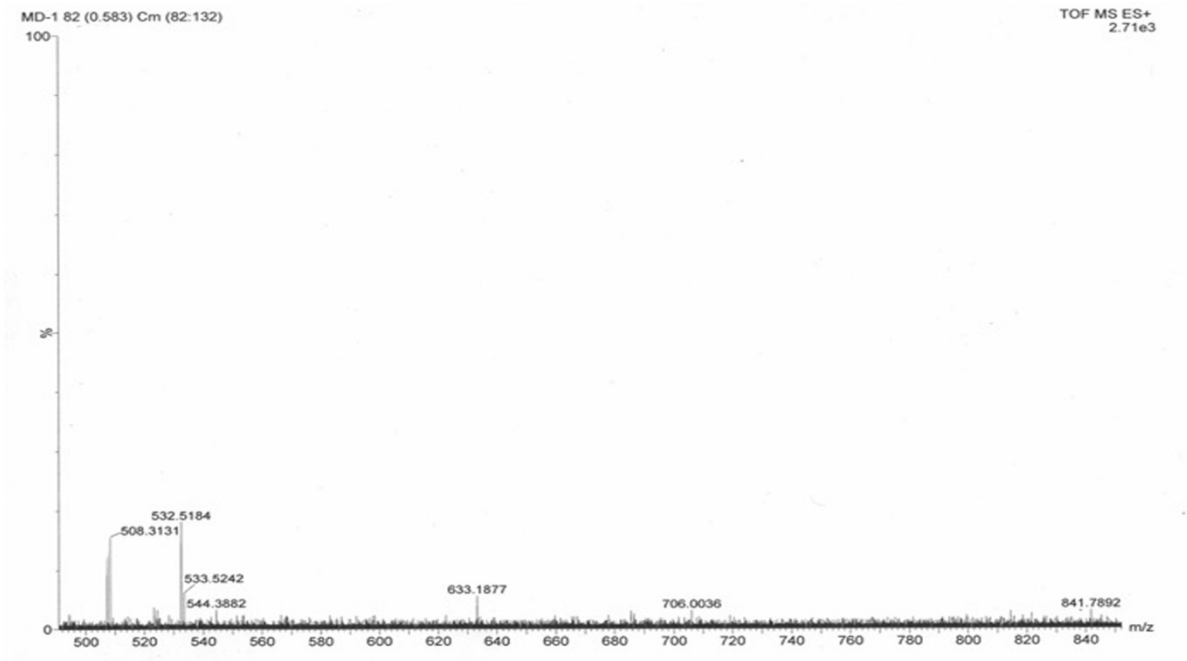


**Figure 2C**
***ESI-MS spectra of compound Quercetin-3-o-rutinoside in R. damaceana.***

D

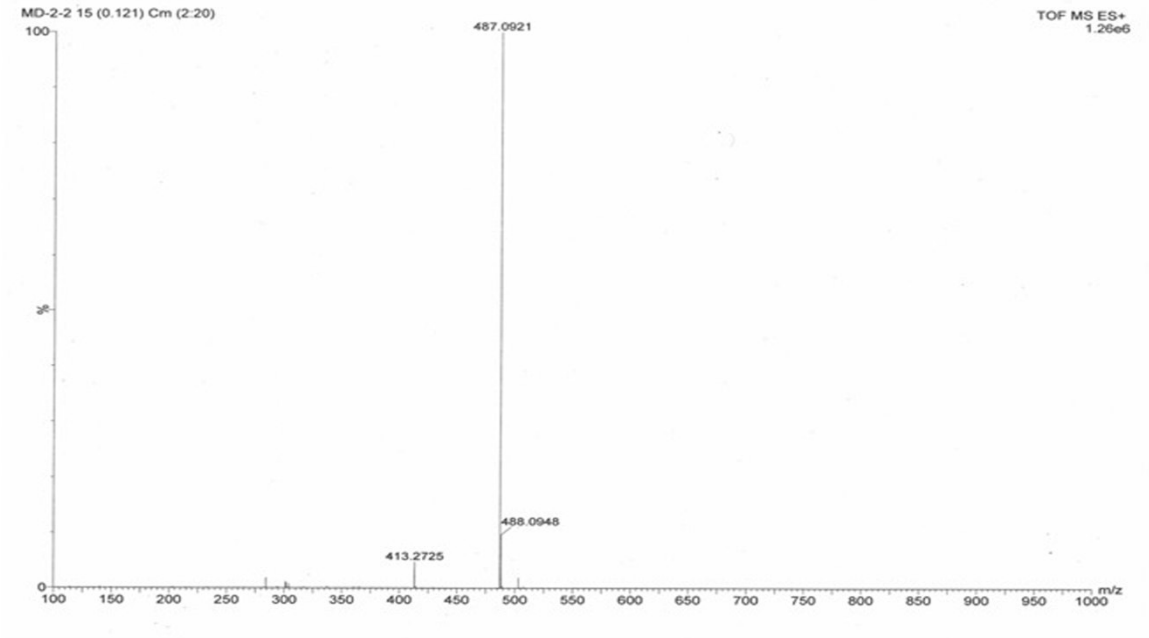


**Figure 2D**

***ESI-MS spectra of compound Quercetin-3-o-glucoside in R. damaceana.***

E

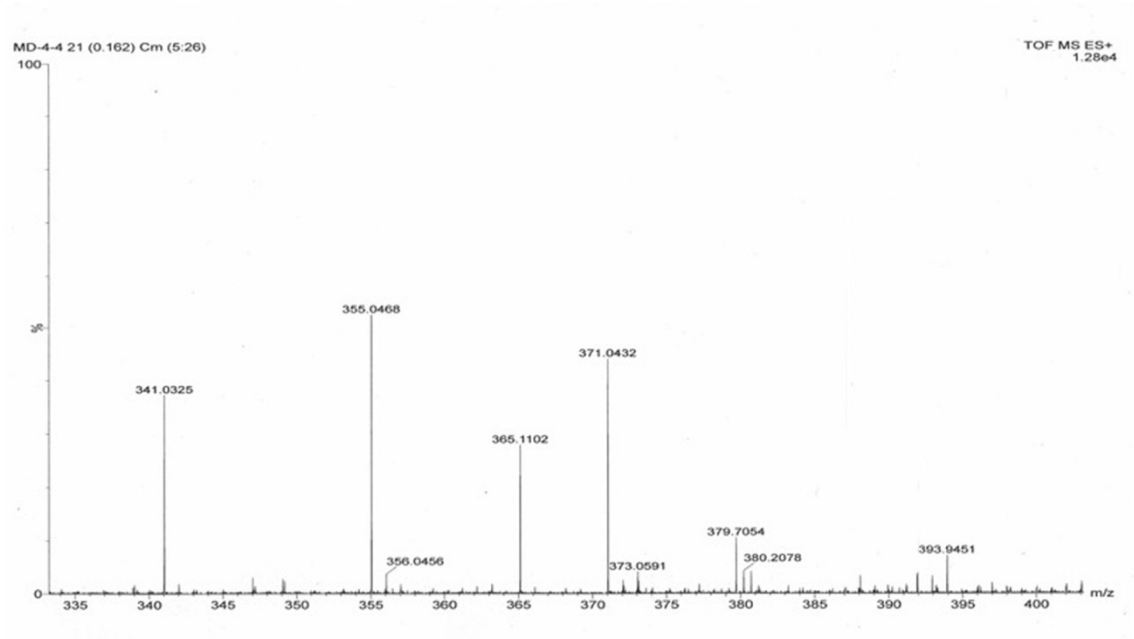


**Figure 2E**

***ESI-MS spectra of compound Quercetin in R. damaceana.***

F

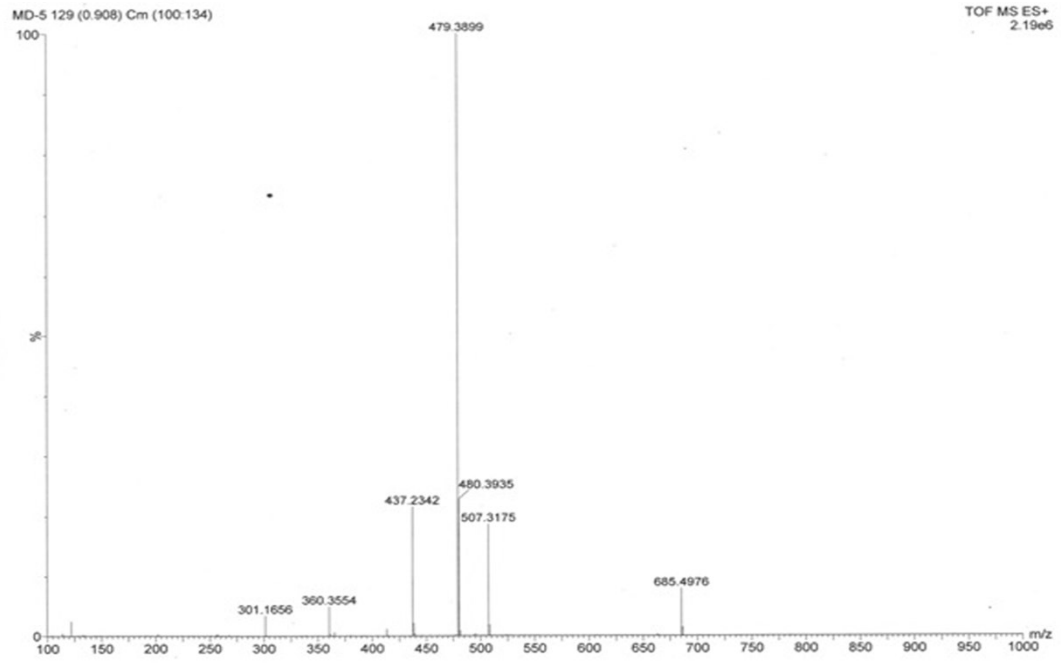


**Figure 2F**

***ESI-MS spectra of compound Oleanolic acid in C. scariosus.***

G

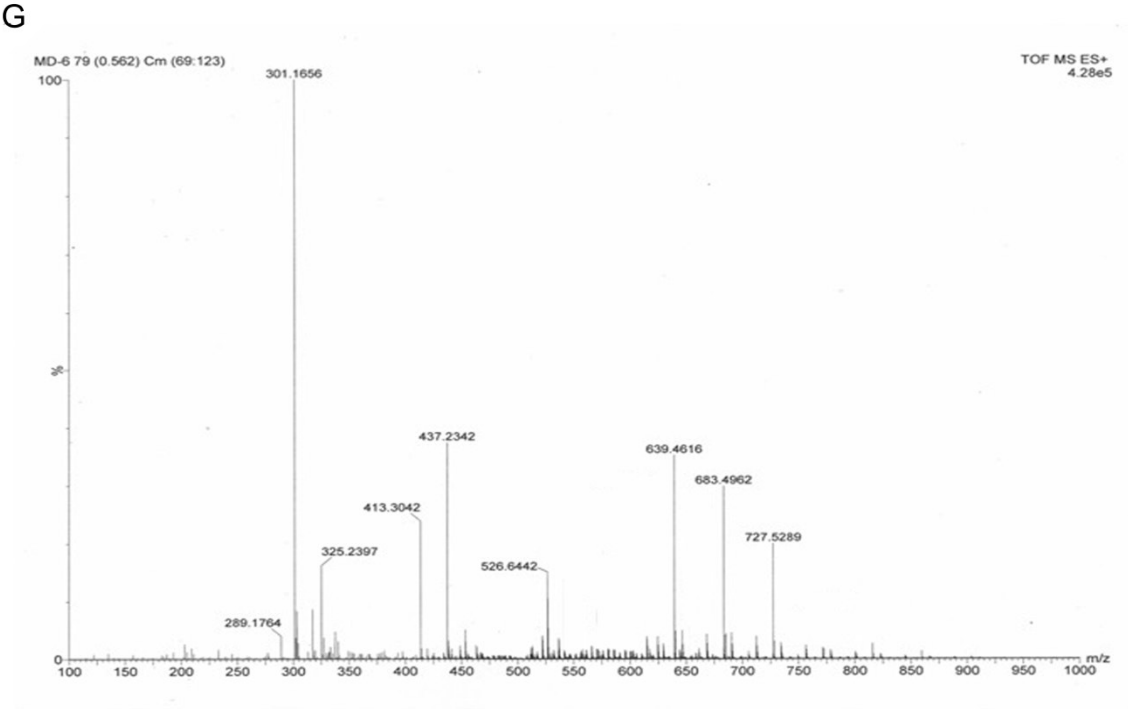


**Figure 2G**

***ESI-MS spectra of compound Linoleic acid in C. scariosus.***

H

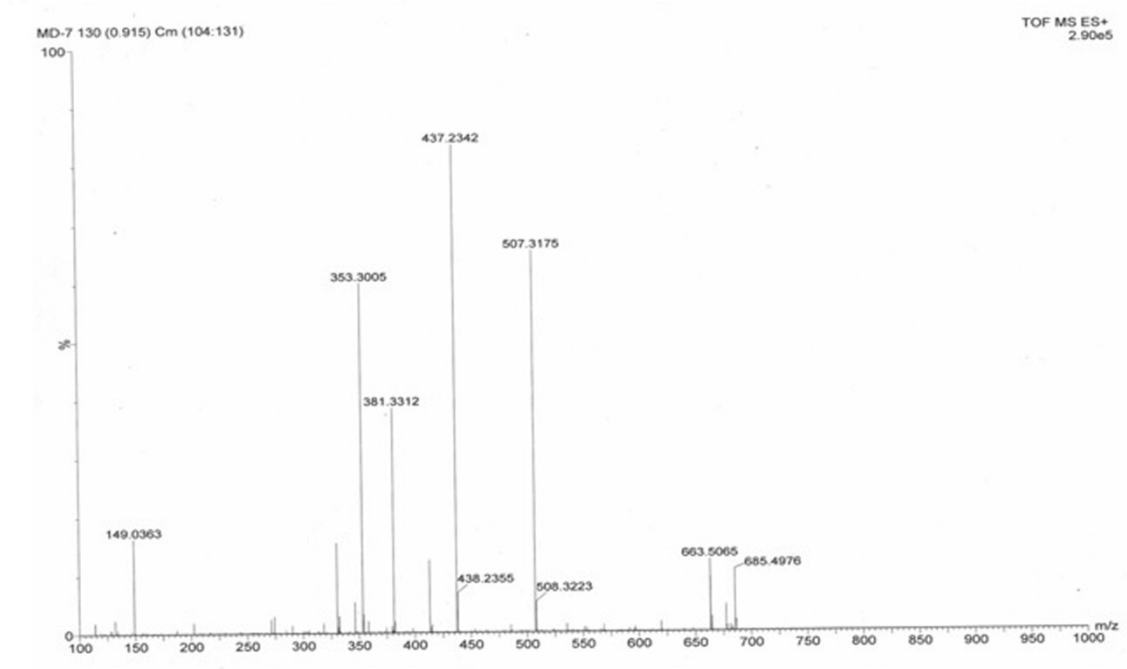


**Figure 2H**
***ESI-MS spectra of compound β Sitosterol in C. scariosus.***

## CONCLUSION

In the present study it was observed that the presence of various effective phytochemicals along with high phenolic content of three active fractions of *C. icosandra, R. damascena* and *C. scariosus* imply them as a potential source of natural antioxidant with considerable activity and no toxicity.

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