Asian Pacific Journal of Tropical Biomedicine

Journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.239430

Phytochemical investigation, antioxidant and wound healing activities of *Citrullus colocynthis* (bitter apple)

Sateesh Chandra Gupta[1], Tusha Tripathi[1], Shravan Kumar Paswan[2], Annie G. Agarwal[1], Chandana V. Rao[2], Om P. Sidhu[1]✉

[1]*Department of Phytochemistry, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, UP, India*

[2]*Department of Pharmacology, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, UP, India*

ARTICLE INFO

Article history:
Received 12 April 2018
Revision 21 May 2018
Accepted 28 July 2018
Available online 23 August 2018

Keywords:
Metabolite profiling
Citrullus colocynthis
GC-MS
Antioxidant
Wound healing

ABSTRACT

Objective: To undertake metabolite profiling of various plant parts of *Citrullus colocynthis*, and assess antioxidant and wound healing activities of fractions for therapeutical applications. **Methods:** Extracts from leaves, stem, root, fruit pulp and seeds were analyzed using gas chromatography-mass spectrometry and high performance liquid chromatography. Variation in antioxidant potential was assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The extract with highest antioxidant potential was subjected on *in-vivo* wound healing activity using excision wound model. **Results:** Metabolite profiling of *Citrullus colocynthis* identified 70 chemically diverse metabolites from different plant parts by using a combination of GC-MS and HPLC. Concentration of colocynthin, a principal active secondary metabolite, ranged from 3.15 mg/g dry weight to 242.00 mg/g dry weight, the lowest being in leaves and highest in fruit pulp. DPPH radical scavenging activity of free radical (IC_{50}) ranged from 196.44 μg/mL in fruit pulp to 413.33 μg/mL in leaves tissues. Significant wound contraction and increase in hydroxyproline content of granulation tissue were observed with ointment formulated from methanolic extract of fruit pulp. **Conclusions:** The study indicates that the methanol extract of *Citrullus colocynthis* fruit pulp when applied topically may promote wound contraction in rat model attributable to the accumulation of colocynthin. The high quantity of colocynthin (242.00 mg/g dry weight) and substantial concentration of 2,4-di-*tert* butyl phenol (3.2%), squalene (4.2%) and δ-tocopherol (2.5%) make this plant to provide new opportunities for development of medicinal, nutraceutical and dietary supplements with optimized functionality.

1. Introduction

Citrullus colocynthis (*C. colocynthis*) Schrad. (Cucurbitaceae) generally known as bitter apple is important traditional plant with a number of phyto-neutraceutical applications[1]. Earlier workers have reported phytochemical constituents such as cucurbitane glycosides, flavonoids, tannin, alkaloids and saponin from different plant parts of *C. colocynthis*[1,2]. *C. colocynthis* has been reported to possess

✉Corresponding author: Om P. Sidhu, Principal Scientist, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India.
Tel: +91-0522-2297940
Fax: 0522-2205847
E-mail: ompsidhu@nbri.res.in

Foundation project: This work is supported by Council of Scientific and Industrial Research (CSIR), New Delhi.

How to cite this article: Gupta SC, Tripathi T, Paswan SK, Agarwal AG, Rao CV, Sidhu OP. Phytochemical investigation, antioxidant and wound healing activities of *Citrullus colocynthis* (bitter apple). Asian Pac J Trop Biomed 2018; 8(8): 418-424.

therapeutic activities against various human ailments[3,4]. Medicinal significance of *C. colocynthis* is due to the existence of colocynthin, cucurbitacin E-2-*O*-glucoside in fruit pulp of this plant[5,6]. Chemical profiling of *C. colocynthis* is limited to colocynthin and fatty acid content[1,7].

Wound healing is a complicated process initiated for restoration of the function and integrity of the tissue damaged by an injury[8]. Screening of potential wound healing phytoconstituents from selected plants is one of the emerging areas in ethanopharmacological sciences.

No systematic work has been done so far on metabolite profiling, antioxidant and wound healing activities of different parts of *C. colocynthis*. Literature survey revealed that antioxidant and wound healing property of the extracts with respect to colocynthin content of this plant has not been studied so far. Metabolite profiling of leaves, stem, root, fruit pulp and seeds was done using analytical techniques such as GC-MS, and HPLC for qualitative and quantitative estimation of metabolites for therapeutic applications. The study also aims to investigate 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and wound healing properties of *C. colocynthis* extracts for healthcare and potential commercial applications.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals utilised during this experiment were of analytical grade. The solvents used in the extraction of metabolites, *n*-hexane and methanol were procured from SD Fine Chemicals Private Limited, Mumbai, India. HPLC grade solvents were procured from Merck Germany. Reagent for GC-MS, methoxyamine-HCL, pyridine and MSTFA, a standard of elaterinide (colocynthin) for HPLC analysis and DPPH for antioxidant assay and other chemicals were procured from Sigma-Aldrich India.

2.2. Plant samples and extraction of metabolites

Leaves, stem, root, fruit pulp and seeds were collected from mature plants of *C. colocynthis* growing at Rajasthan, India. The samples were dried until a constant weight was obtained. For non-targeted metabolite profiling, 1 g of each sample was extracted with *n*-hexane followed by methanol and analysis was performed by GC-MS and HPLC-PDA reported earlier[9].

2.3. Analysis of metabolites using GC-MS

A TMS derivative of semi-polar as well as non-polar metabolites of root, stem, leaves, fruit pulp and seeds samples was prepared for gas chromatography-mass spectrometry analysis. The reaction mixture was prepared by dissolving 5 mg of the extracts in 50 μL of methoxyamine-HCL solution in GC grade pyridine (20 mg/mL). Mixtures were shaken for 2 h at 40 ℃ using thermo mixer Comfort (Eppendorf India Ltd.) and added 70 μL of *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide. Shaking was continuing for next thirty min at 40 ℃. GC-MS analysis was done using Thermo Trace GC Ultra coupled with Thermo Fisher DSQ Ⅱ mass spectrometers. Chromatographic separations of metabolites were carried out using thermo TR50 column. Xcalibur software was used to process the chromatographic and mass spectrometric data. The oven temperature of gas chromatography was maintained at 70 ℃ for 5 min, then gradually raised at the rate of 5 ℃/min, 70 ℃ to 310 ℃ and maintained for 5 min. The sample was injected in the split mode (splitting ratio of 1:16). The flow rate of carrier gas (Helium) was 1 mL/min. The MS detector was run in the electron impact (EI) mode, with electron energy of 70 eV. The resulting GC-MS profile was analyzed using WILLY and NIST mass spectral library[9].

2.4. HPLC analysis

HPLC-photo diode array analysed the methanolic extracts of leaves, stem, root, fruit pulp and seeds samples from *C. colocynthis* that were completed on a Waters liquid chromatography with Waters 600 controller, Waters Delta 600 solvent delivery system attached along with Waters In-Line degasser AF, Sample injector (Rheodyne 7125) with 20 microlitre loop and a Waters 2996 UV- Vis PDA detector. Colocynthin was detected at 237 nm using an isocratic mixture comprised of methanol: water (50:50) at flow rate of 1 mL/min using a Waters XTerra RP C18 column (4.6 mm × 150 mm). The results were presented on a percent weight basis and were quantified using external standards of colocynthin.

2.5. Antioxidant potential using DPPH assay

Methanolic extracts of *C. colocynthis* were evaluated for their DPPH radical scavenging potential as described earlier[10]. A 50 microlitre of sample solution was added to 5 millilitre of 0.004% methanolic 1, 1-diphenyl-2-picrylhydrazyl and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm. DPPH assay (IC_{50}) values showed the concentration of compounds to scavenge 50% of DPPH. The assay was taken out in triplicate using ascorbic acid as standard.

2.6. Wound healing and dermal irritation studies

Healthy Wistar rats (weight 120-160 g) of either sex, without

previous treatment were used in this experiment. Experimental rats were shifted in polypropylene cages maintained at (22±2) ℃ with light and dark cycle of 12 h. All animal studies were conducted according to Institutional Animal Ethics Committee (1732/GO/Re/S/13/CPCSEA) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. Rats were classified into 4 groups containing 6 animals in every group. The control rats (group Ⅰ) received normal saline and served as negative control, the experimental rats (group Ⅱ) served with standard ointment (Betadine) and served as positive control. Test group Ⅲ and Ⅳ were treated with the methanolic extract of *C. colocynthis* fruit pulp ointment (5% and 10% w/w).

2.6.1. Preparation of ointments

The test samples for topical application were prepared in an ointment base (vehicle) consisting of white soft paraffin, hard paraffin, cetostearyl alcohol and wool fat. The methanolic extracts of *C. colocynthis* fruit pulp formulation 5% and 10% were then added to the vehicle (1 g) respectively.

2.6.2. Acute dermal irritation test

The study was taken out abiding to OECD guidelines 402: acute dermal irritation and corrosion[11]. A total of 5 cm^2 on dorsal hair of animals was removed 24 h before application of the sample. Animals were separately treated with simple ointment, 5% and 10% methanolic extract of *C. colocynthis* fruit pulp formulated ointment. The skins were noticed for inflammation, swelling, redness and other related symptoms following the method described earlier after 4 hours of the treatment[12].

2.6.3. Excision wound model

Experimental rats were anaesthetized with slight vapour inhalation of diethyl ether in anaesthesia chamber. The skin was excised from the sterile dorsal marked area from shaved dorsal surface of the experimental rats to get a wound measuring about 8 mm diameter. These wounds were left open to air and treated with topical application of ointment daily for a period of 16 days. Wounds were left open and ointment was applied topically twice daily for a period of 16 days.

2.6.4. Wound contraction

Wound area was measured at day zero (8 mm^2) and once every 4 days post wounding. Excision wound margins were traced using transparent paper from which the wound surface area was obtained. The measured surface area at different days of post wound creation was employed to calculate the percentage of wound contraction, taking the initial size of the wound as 100%, according to Shenoy *et al*.[13].

% Wound contraction = (initial wound size-specific day wound size)/ initial would size × 100

2.6.5. Estimation of hydroxyproline content

In order to determine hydroxyproline content, fresh granulation tissues of the rats from each experimental group were removed and weighed on the 16th post wounding day. Granulation tissues were dried at 50-60 ℃ in an oven and weighed. The dried tissues were hydrolyzed by 6N HCl at 120 ℃ for 4 h and neutralized then subjected to chloramines T-oxidation. The reaction was stopped by addition of perchloric acid and colour was produced with Ehrlich reagent. The hydroxyproline was determined by spectrophotometric technique as indicated by Goyal *et al*.[14].

2.7. Statistical analysis

The statistical significance (P<0.05) for the relative peak area of GC-MS identified metabolites was determined by one-way ANOVA *post hoc* Bonferroni multiple comparison test (SPSS 16, USA) between five parts of the plant. The statistical significance (P<0.05) of the wound healing assay was analyzed using one way ANOVA followed by Student-Newman-Keuls t-test. For multivariate principal component analysis (PCA), the HPLC and GC-MS data of metabolites were analysed using Unscrambler X Software package (Version 10.0.1, CAMO, USA).

3. Results

Five plant parts *viz*.: leaves, stem, root, fruit pulp, and seeds of *C. colocynthis* were investigated for metabolite profiling using GC-MS and HPLC. Hexane and methanol soluble crude content in different plant parts was shown in Figure 1. Hexane soluble content ranged from 0.20% to 7.94% with an average of 2.16%. The methanol soluble content ranged from 1.27% to 11.3%, the lowest being in seeds and highest in fruit pulp (Figure 1).

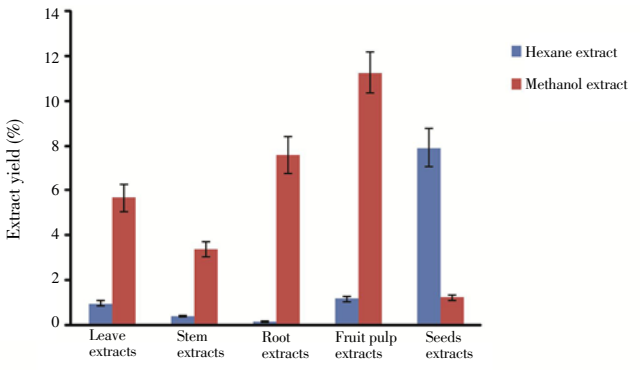

Figure 1. Percent yield of hexane and methanol extract of *C. colocynthis*.

Table 1

Qualitative and quantitative variability in semi-polar and non-polar metabolites among different plant parts of *C. colocynthis*.

Metabolites	Plant Parts				
	Root	Stem	Leaves	Fruit pulp	Seeds
Lactic acid	0.10±0.02b^{e}	0.22±0.02^{e,f}	0.74±0.05^{h,i}	1.24±0.12^{j}	0.14±0.02
Glycerol	1.50±0.10^{a,b,e}	4.56±0.38^{f,g}	4.63±0.45^{h,i}	2.50±0.19^{j}	1.28±0.10
Succinic acid	0.50±0.04^{a,b,e,d}	0.67±0.05^{e,f,g}	0.28±0.03^{h}	1.31±0.10^{j}	0.21±0.03
Malic acid	5.56±0.37^{a,b,e,d}	3.52±0.24^{e,f}	0.81±0.07^{h,i}	2.98±0.27^{j}	3.52±0.26
Xylitol	0.32±0.03^{a,b}	7.48±0.54^{f,g}	7.79±0.78^{h,i}	0.52±0.04	0.30±0.03
Proline	0.17±0.02^{a,b,e}	1.51±0.11^{e,f,g}	ND	1.04±0.10^{h,j}	0.21±0.02^{i}
Glucitol	0.19±0.02^{a,b,d}	5.58±0.31^{e,f,g}	10.39±0.68^{h,i}	0.32±0.03^{j}	ND
Fructose-O-methyloxime	21.77±2.02^{a,b,e}	11.58±0.70^{e,f,g}	2.78±0.18^{h,i}	25.50±2.06^{j}	19.16±1.14
Glucose-O-methyloxyme	27.26±1.62^{a,b,e,d}	4.39±0.43^{f,g}	4.01±0.50^{h,i}	11.22±1.08^{j}	13.52±0.95
Myo-Inositol	2.20±0.25^{a,e,d}	2.99±0.22^{e,f,g}	2.17±0.13^{h,i}	4.70±0.45	4.44±0.32
Sucrose	9.66±0.66^{a,b,e,d}	1.74±0.11^{e,f,g}	0.14±0.02^{h,i}	4.95±0.42^{j}	15.72±1.01
Turanose	2.42±0.23^{a,b}	25.18±1.35^{e,f,g}	32.72±1.91^{h,i}	0.59±0.05	1.13±0.02
Caprylic acid	0.08±0.01^{a,d,e,d}	0.06±0.00^{e,f,g}	0.05±0.01^{i}	0.05±0.01^{j}	ND
2,4 di-*tert* butyl phenol	0.51±0.04^{b,d}	0.63±0.05^{e,g}	0.44±0.03^{h,i}	3.20±0.23^{j}	1.04±0.06
Hexadecene	0.14±0.01^{a,b}	0.24±0.02^{e,f,g}	0.80±0.07^{h,i}	0.16±0.02	0.16±0.02
Lauric acid	0.17±0.02^{a,b,e,d}	0.13±0.02^{f,g}	0.12±0.01^{h,i}	ND	0.28±0.03^{j}
Myristic acid	1.09±0.08^{a,e,d}	0.79±0.06^{e,f,g}	1.04±0.13^{h,i}	0.51±0.05^{j}	1.90±0.21
Palmitic acid	24.34±1.51^{a,b,d}	19.27±1.05^{e,f,g}	11.43±0.92^{h,i}	22.16±1.17^{j}	27.55±1.79
Phytol	ND	0.48±0.02^{a,e,f,g}	2.41±0.14^{b,h,i}	0.34±0.04^{ej}	ND
Margaric acid	1.22±0.08^{a,b,e,d}	0.88±0.07^{e,f}	0.46±0.04^{h,i}	0.61±0.03^{j}	0.91±0.07
Stearic acid	7.30±0.05^{a,d}	8.95±0.80^{e}	7.57±0.47	8.21±0.50	8.59±0.58
Linoleic acid	13.92±1.01^{a,b,e,d}	6.89±0.77^{e,f,g}	4.63±0.28^{h,i}	9.98±0.67^{j}	33.92±1.65
Linolenic acid	5.99±0.57^{a,b}	1.64±0.09^{f,g}	1.52±0.17^{h,i}	5.18±0.43^{j}	6.29±0.44
Eicosatrienoic acid	0.61±0.06^{a,e,d}	0.99±0.10^{e,f}	0.50±0.04^{h,i}	2.15±0.22^{j}	1.08±0.08
Behenyl alcohol	0.60±0.06^{b,e,d}	0.46±0.05^{e,f,g}	0.31±0.03^{h,i}	0.98±0.14^{j}	0.15±0.02
Heneicosylic acid	0.30±0.02^{a,b,e}	0.22±0.02^{e,f,g}	ND	0.35±0.03^{h}	0.33±0.04^{i}
Behenic acid	1.61±0.15^{a,b,e}	2.71±0.27^{e,f,g}	1.03±0.06^{h}	5.79±0.34^{j}	1.39±0.13
Thymol-glucopyranoside	0.16±0.02^{a,b,e,d}	2.75±0.22^{e,f,g}	1.16±0.12^{i}	0.89±0.08^{j}	ND
Heptacosane	0.04±0.00^{a,b,e,d}	0.46±0.04^{f,g}	0.50±0.03^{i}	0.55±0.06^{j}	ND
Lignoceric acid	1.13±0.09^{a,b,e,d}	1.33±0.12^{e,f,g}	0.54±0.03^{h,i}	0.89±0.04^{j}	0.36±0.03
Dotriacontane	0.08±0.01^{a,b,e,d}	1.00±0.15^{g}	1.10±0.16^{h,i}	0.88±0.07^{j}	ND
Squalene	0.64±0.07^{b,e,d}	0.79±0.08^{e,f,g}	4.16±0.42^{h,i}	ND	0.18±0.01^{j}
δ -Tocopherol	ND	ND	2.50±0.33^{b,e,h,i}	ND	ND
Tetratetracontane	ND	1.20±0.15^{a,f,g}	1.26±0.09^{b,h,i}	0.94±0.06^{e,j}	ND
Octacosyl alcohol	0.20±0.02^{a,b,e,d}	0.96±0.09^{e,f,g}	2.13±0.21^{i}	1.93±0.12^{j}	ND
Hydroxycholesterol	ND	ND	ND	6.96±0.42^{e,f,h,j}	ND
Triacontanol	0.18±0.02^{a,b,e,d}	1.43±0.15^{e,f,g}	4.40±0.49^{h,i}	ND	ND
Campesterol	0.28±0.03^{b,e}	0.27±0.02^{e,f}	ND	1.07±0.12^{h,i,j}	0.27±0.02
Stigmasterol	ND	ND	1.26±0.20^{b,e}	0.15±0.02^{e,f,h,j}	0.43±0.04^{d,g,i}
β -Sitosterol	ND	ND	ND	0.53±0.07^{e,f,h,j}	2.50±0.20^{d,g,i}
Ergosta-7,22-dien-3 β -ol	18.43±0.95^{a,b,e,d}	21.11±1.66^{e,f,g}	8.18±0.56^{i}	9.17±0.56^{j}	1.21±0.08
5 α -Stigmastan-3 β -ol	10.02±1.01^{a,b,e,d}	14.52±1.03^{f,g}	16.86±1.05^{h,i}	5.53±0.64^{j}	2.76±0.25
Colocynthin*	76.71±7.57^{a,b,e,d}	9.93±0.91^{e,f,g}	3.15±0.35^{h,i}	242.00±20.76^{j}	58.54±4.76

Mean values ± SD of relative peak percentage area; * = quantified by HPLC (mean values ± SD mg/g of dry weight); ND = not detected; a, b, c, d, e, f, g, h, i, j denote statistical significance $P< 0.05$ *i.e.* a= Root *vs* Stem; b= Root *vs* Leaves; c= Root *vs* Fruit pulp; d= Root *vs* Seeds; e= Stem *vs* Leaves; f= Stem *vs* Fruit pulp; g= Stem *vs* Seeds; h= Leaf *vs* Fruit pulp; i= Leaf *vs* Seeds; j= Fruit pulp *vs* Seeds.

3.1. Metabolic profiling of hexane and methanolic extracts using GC-MS

Non-targeted metabolite profiling of hexane and methanolic extracts of all the five plant parts was carried out using GC-MS with five replicates of every sample. GC-MS based metabolite profiling of hexane extracts identified thirty chemically different metabolites comprising hydrocarbons, alkyl carboxylic acids, fatty acids, fatty alcohols, phenol, triterpene, vitamin and sterols (Figure 2). One-way ANOVA indicated that concentration of metabolites varied significantly among various parts of *C. colocynthis* (Table 1). Linoleic and palmitic acid were detected as the major metabolites of hexane extracts in seeds. Percent peak area of palmitic acid ranged from (11.43 ± 0.92) to (27.55 ± 1.79), the lowest being in leaves and highest in seeds. Linoleic acid content ranged from (4.63 ± 0.28)% in leaves to (33.92 ± 1.65)% in seed with an average of (13.86 ± 0.97)% in fruit pulp, respectively. The concentration of 2,4-di-*tert* butyl phenol ranged from (0.44 ± 0.03)% to (3.20 ± 0.23)%, which was lowest in leaves and highest in fruit pulp (Table 1).

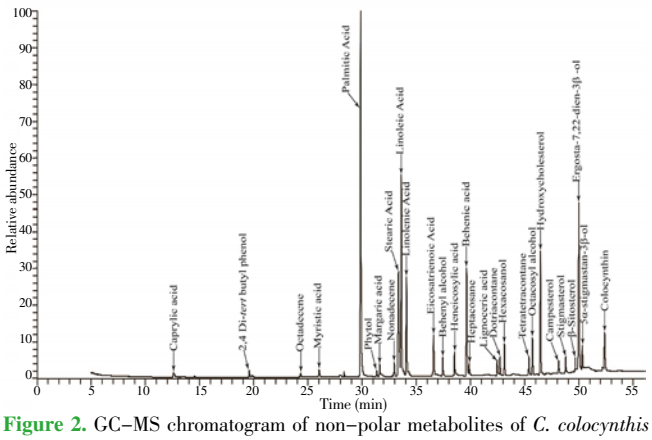

Figure 2. GC-MS chromatogram of non-polar metabolites of *C. colocynthis* fruit pulp.

Twelve chemically diverse metabolites comprising amino acids, organic acid, cucurbitane glycoside, sugars, sugar alcohols and inositol were characterized in methanolic extracts of five various parts of *C. colocynthis*. The concentration of metabolites varied significantly among various plant parts (Table 1). Glucose, fructose and sucrose were found to be the dominant metabolites in semi-polar extracts of *C. colocynthis*.

3.2. HPLC analysis

Cucurbitacin E 2-*O*- β -D-glucopyranoside (colocynthin) from methanolic extracts of leaves, stem, root, fruit pulp and seeds of *C. colocynthis* was determined using HPLC. The concentration of colocynthin varied significantly among different plant parts. The colocynthin content was (3.15 ± 0.35) mg/g dry weight in leaves, (9.93 ± 0.91) mg/g dry weight in stem, (58.54 ± 4.76) mg/g dry weight in seeds, (76.71 ± 7.57) mg/g dry weight in root and (242.00 ± 20.76) mg/g dry weight in fruit pulp, respectively. Principal component analysis from the samples of five plant parts could be classified into three groups, *viz*. stem and leaves (group Ⅰ), root and seeds (group Ⅱ) and fruit pulp (group Ⅲ) (Figure 3A & B).

3.3. DPPH radical scavenging activity

DPPH radical scavenging potential of methanolic extracts of leaves, stem, root, fruit pulp and seeds of *C. colocynthis* showed significant differences amongst plant parts. The methanolic extract of fruit pulp showed higher free radical scavenging activity by DPPH assay [IC_{50}: (196.44 ± 17.78) μg/mL] as compared to that of leaves (413.33 ± 39.78), stem (374.68 ± 38.22), root (288.46 ± 29.07) and seeds (314.66 ± 31.66) μg/mL, respectively. The relatively high concentration of colocynthin in methanolic extract of fruit pulp accounted for the DPPH radical scavenging potential of *C. colocynthis*.

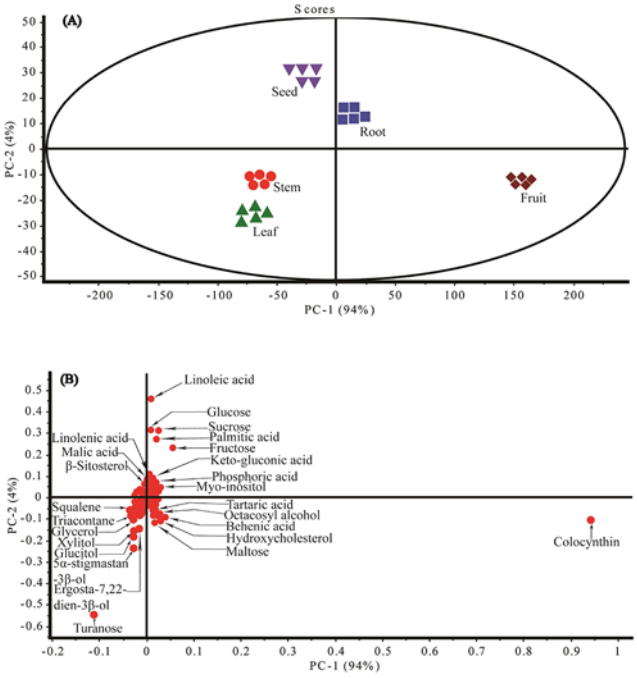

Figure 3. PCA score plot of non-polar and semi-polar extract of *C. colocynthis* (A) and loading plot (B).

3.4. Wound healing activity

The methanol extracts of *C. colocynthis* fruit pulp in the form of ointment at two different concentration (5% w/w and 10% w/w) did not show any type of irritation, swelling, noticeable inflammation and any other change on skin. The ointment consisted of 5% and 10% of methanolic extracts of *C. colocynthis* fruit pulp applied regularly for a period of 16 days significantly minimized the area of open wounds in comparison with control group Ⅰ. The observation revealed a better healing pattern as percentage contraction in wound area of the methanolic extract of *C. colocynthis* fruit pulp (10%) treated group, group Ⅳ, (25.64%, 55.08%, 74.35% to 84.61%) than that of the control group, group Ⅰ (14.28%, 51.87%, 61.53% to 74.72%) on 4th, 8th, 12th and 16th day respectively (Figure 4). Group Ⅲ and Ⅳ demonstrated significant reduction in wound areas on the day 16 after wounding compared to vehicle-treated control group Ⅰ.

3.5. Effect of methanolic extract of C. colocynthis fruit pulp on hydroxyproline content

The fresh and dry weight of tissue treated with standard drug betadine, 5% and 10% of ointment was considerably higher as compared with control group. Hydroxyproline content of Betadine and 10% formulated ointment was significantly higher [(5.89±0.15) mg/g and (4.72±0.11) mg/g] as compared to control group animals [(2.13±0.05) mg/g] (Table 2).

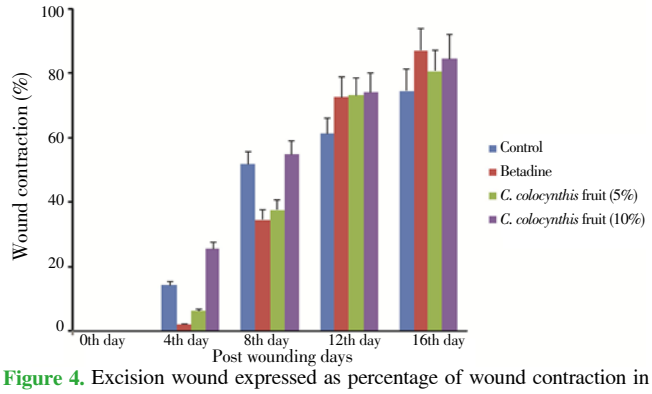

Figure 4. Excision wound expressed as percentage of wound contraction in control and treated groups.

Table 2

Effect of formulated ointments from methanolic extract of *C. colocynthis* fruit pulp on hydroxyproline content in granulation tissue.

Samples	Fresh weight (g)	Dry weight (g)	Hydroxyproline (mg/g)
Control	4.21±0.09*	0.72±0.01*	2.13±0.05*
Betadine	8.09±0.24**	1.61±0.04**	5.89±0.15**
C. colocynthis fruit pulp (5%)	6.10±0.10*	1.95±0.07*	3.23±0.09**
C. colocynthis fruit pulp (10%)	7.32±0.18**	1.33±0.03**	4.72±0.11**

Values were expressed as mean ± SEM; One Way ANOVA followed by Student-Newman-Keuls *t*-test value denoted significance at $^{*}P<0.05$, $^{**}P<0.01$.

4. Discussion

In GC-MS and HPLC based metabolite profiling, significant alterations were seen in levels of phyto-neutraceuticals important metabolites *viz*. colocynthin, 2,4-di-*tert* butyl phenol, squalene and δ –tocopherol among the five different plant parts.

A relatively higher concentration of δ-tocopherol and squalene in leaves suggests nutritional and healthcare properties[15,16]. A substantial quantity of colocynthin in fruit pulp (242.00 mg/g dry weight), root (76.7 mg/g dry weight) and seeds (58.5 mg/g dry weight), and 2,4-di-*tert* butyl phenol (3.2%), squalene (4.16%) and δ-tocopherol (2.5%) in leaves makes the taxa as a new potential source of healthcare products. However, the relative percent peak area of 2,4-di-*tert* butyl phenol, squalene and δ-tocopherol was higher in leaves as compared to that of other plant parts investigated. Phenols are potent antioxidant compounds which can be of commercial importance for the nutraceutical industry. Earlier, Varsha *et al.* have reported that 2,4-di-*tert*-butyl phenol is an fungicidal with antioxidant properties[17]. Colocynthin, cucurbitacin E-2-*O*-glucoside that is the main active principle present in the pulp of *C. colocynthis* has been reported to have cathartic, antihistaminic, anticholinergic, negative chronotropic and negative inotropic activities[6].

DPPH radical scavenging of methanolic extracts varied significantly among different parts of *C. colocynthis*. However, the highest radical scavenging potentioal was found in fruit pulp as compared to the other parts. Indeed, colocynthin content was comparatively higher in fruit pulp of *C. colocynthis* as compared to that of the other plant parts. Therefore, the colocynthin content may be one of the constituents of the fruit pulp of *C. colocynthis* responsible for the antioxidant activity.

The wound healing study showed that the ointments from the methanolic extract of *C. colocynthis* resulted in an improved wound closure rate and significant reduction in healing time as compared with control group Ⅰ. These results enhanced wound healing progression and noticeable wound margin hydration resulting from skin regeneration. The transformation activity of the ointment that increased statistically significant enhanced cellular proliferation against wound healing. The estimation of hydroxyproline indicates that a significant increase in hydroxyproline content of granulation tissue is responsible for synthesis of collagen, which is the predominant protein responsible for the wound healing[18].

In present study, GC-MS and HPLC based metabolite profiling was applied to various parts of *C. colocynthis* in order to analyse metabolites of potential dietary supplements or nutraceutical applications could be explored as potent free radical scavenging activity. The chemically diverse metabolites were detected in semi-polar and non-polar extracts of different parts of *C. colocynthis*. Colocynthin content was higher in fruit pulp and lowest in leaves in the methanolic extracts of *C. colocynthis*. The results show that the methanolic extract of fruit pulp has a significant effect on DPPH scavenging free radicals *in vitro*, wound healing and hydroxyproline content in experimental animals. Earlier workers have reported that methanolic and aqueous methanolic extracts of leaves and fruit of *C. colocynthis* possess antioxidant activity *in vitro*[19]. The results of wound healing study strongly indicated that 10% methanolic extract of *C. colocynthis* fruit pulp may be a potential candidate for wound care because of its positive influence on various phases of the healing process. DPPH radical scavenging and wound healing activities of fruit pulp extracts may be attributed to the higher content of colocynthin in *C. colocynthis*. A very high concentration of colocynthin in fruit pulp and the substantial quantity of 2,4-di-*tert* butyl phenol, squalene and δ-tocopherol in leaves make the taxa as a new potential source of healthcare products.

Conflict of interest statement

The authors have no conflict of interests.

Acknowledgements

Financial support from the Council of Scientific and Industrial

Research (CSIR), New Delhi is gratefully acknowledged. The authors are thankful to Director CSIR-NBRI, Lucknow, for providing the needed facilities.

References

[1] Pravin B, Tushar D, Vijay P, Kishanchnad K. Review on *Citrullus colocynthis*. *Int J Res Pharm Chem* 2013; **3**(1): 46-53.

[2] Rahimi R, Amin G, Ardekani MRS. A review on *Citrullus colocynthis* Schrad.: From traditional Iranian medicine to modern phytotherapy. *J Altern Complement Med* 2012; **18**: 551-554.

[3] Kim MG, Lee SE, Yang JY, Lee HS. Antimicrobial potentials of active component isolated from *Citrullus colocynthis* fruits and structure-activity relationships of its analogues against foodborne bacteria. *J Sci Food Agric* 2014; **94**: 2529-2533.

[4] Gurudeeban S, Satyavani K, Ramanathan T. Bitter apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. *Asian J Plant Sci* 2010; **9**(9): 394.

[5] Yoshikawa M, Morikawa T, Kobayashi H, Nakamura A, Matsuhira K, Nakamura S, et al. Bioactive saponins and glycosides. XXⅦ. Structures of new cucurbitane-type triterpene glycosides and antiallergic constituents from *Citrullus colocynthis*. *Chem Pharm Bull* 2007; **55**(3): 428-434.

[6] Shekarchi M, Ramezany F, Shams Ardekani MR, Lamshöft M, Rastegar H, Eftekhari M, et al. An improved HPLC method for determination of colocynthin in colocynth. *Res J Pharmacogn* 2015; **2**(4): 39-48.

[7] Gurudeeban S, Ramanathan T, Satyavani K, Dhinesh T. Antimicrobial effect of coastal medicinal plant *Citrullus colocynthis* against pathogenic microorganisms. *Afr J Pure Appl Chem* 2011; **5**(5): 119-122.

[8] Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing-exploring medicinal plants of India. *J Ethnopharmacol* 2007; **114**: 103-113.

[9] Bhatia A, Bharti SK, Tripathi T, Mishra A, Sidhu OP, Roy R, et al. Metabolic profiling of *Commiphora wightii* (guggul) reveals a potential source for pharmaceuticals and nutraceuticals. *Phytochemistry* 2015; **110**: 29-36.

[10] Güllüce M, Sökmen M, Daferera D, Ağar G, Özkan H, Kartal N, et al. *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J Agric Food Chem* 2003; **51**(14): 3958-3965.

[11] OECD. *Test No. 402: Acute dermal toxicity. OECD guidelines for the testing of chemicals*. OECD Publishing, Paris; 2017.

[12] Sanwal R, Chaudhary AK. Wound healing and antimicrobial potential of *Carissa spinarum* Linn. in albino mice. *J Ethnopharmacol* 2011; **135**(3): 792-796.

[13] Shenoy RR, Sudheendra AT, Nayak PG, Paul P, Kutty NG, Rao CM. Normal and delayed wound healing is improved by sesamol, an active constituent of *Sesamum indicum* (L.) in albino rats. *J Ethnopharmacol* 2011; **133**(2): 608-612.

[14] Goyal M, Nagori B, Sasmal D. Wound healing activity of latex of *Euphorbia caducifolia*. *J Ethnopharmacol* 2012; **144**(3): 786-790.

[15] Amarowicz R. Squalene: A natural antioxidant? *Eur J Lipid Sci* 2009; **111**(5): 411-412.

[16] Goffman FD, Galletti S. Gamma-linolenic acid and tocopherol contents in the seed oil of 47 accessions from several *Ribes* species. *J Agric Food Chem* 2001; **49**(1): 349-354.

[17] Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A, Nampoothiri KM. 2, 4-Di-*tert*-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. *Int J Food Microbiol* 2015; **211**: 44-50.

[18] Chithra P, Sajithlal G, Chandrakasan G. Influence of *Aloe vera* on the glycosaminoglycans in the matrix of healing dermal wounds in rats. *J Ethnopharmacol* 1998; **59**(3): 179-186.

[19] Hussain AI, Rathore HA, Sattar MZ, Chatha SA, Ahmad F, Ahmad A, et al. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* (L.) from the Pakistani flora. *Ind Crops Prod* 2013; **45**(1): 416-422.