PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC AND FLAVONOIDS CONTENTS AND ANTIOXIDANT ACTIVITIES OF *CITRULLUS COLOCYNTHIS* L. AND *CANNABIS SATIVA* L.

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Abstract. *Citrullus colocynthis* L. and *Cannabis sativa* L. are well known herbs for their curative value and farming applications. Thus, extraction was performed to examine yield, total phenolic, flavonoids content and antioxidant activities for farming exertions. Qualitative analyses were conducted to scrutinize alkaloids, glycosides, terpenoids, flavonoids, flavones, steroids, tannins, phenols, saponins and quantitative analysis for total phenolic and flavonoids content was assessed by Folin-Ciocalteu and aluminium chloride colourimetric method respectively, while antioxidant activities was assessed by using 1, 1-Diphenyl-1-picrylhydrazyl. Results showed that distilled water afforded the maximum extract yield followed by methanol and ethanol thus, coefficient of determination (R^2) of extract yield present a positive correlation compared to root mean square error (RMSE). Phytoconstituents except terpenoids from *C. sativa* and *C. colocynthis* leaves were reported while, in contrast, steroids, tannins and phenols were absent in *C. colocynthis* roots. The methanol derived maximum phenolic contents from *C. sativa* and *C. colocynthis* leaves were 36.42 and 37.69 mg gallic acid equivalent GAE/g respectively. However, total flavonoids registered from *C. sativa* leaves and *C. colocynthis* leaves and roots were 59.03, 50.58 and 43.32 mg quercetin equivalent QE/g respectively. Interestingly, *C. colocynthis* leaves produced the highest flavonoids 119.63 mg QE/g using ethyl acetate extract. DPPH inhibition (%) was high in acetone 55.57, hexane 45.98 and distilled water 35.5% from *C. sativa*, *C. colocynthis* leaves and roots respectively. Our findings suggest that studied plants contain phytochemicals, reasonable quantity of phenol and flavonoids content confer to the potential antioxidant activity responsible for insecticidal properties as safer alternatives of synthetic pesticides.
Keywords: *solvent extraction, biochemical analysis, DPPH inhibition %, insecticidal agents*

Introduction

Plants are a God-given treasure to human beings; they produce a variety of secondary metabolites, i.e., alkaloids, flavonoids, steroids, glycosides, terpenoids, tannins, saponins, quinine and phenols (Jung et al., 2009) and have the best curative potential of pharmaceutical medicines, biocides and biopesticides (Edeoga et al., 2005; Wells et al., 2009). Plant-produced phytochemicals perform distinctive functions in agriculture, veterinary, and pharmaceutical industries (Vasu et al., 2009).

Several plants are used as herbal medicines and in agriculture farming, including *Citrullus colocynthis* and *Cannabis sativa*. *C. colocynthis*, locally known as Tumba, is viny herbaceous plant that grows in desert or sandy soils, inhabitant to North Africa and is scattered in northern Africa, eastward Sahara, Morocco, Egypt, Sudan, Iran, Afghanistan, Pakistan, India and other tropical areas (Dane et al., 2007; Pravin et al., 2013). It belongs to the family Cucurbitaceae has, gained attention as a natural botanical

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insecticide; its insecticidal activity has been evaluated against several insect pests' species (Soam et al., 2013). Moreover, extract obtained by dichloromethane from *C. Colocynthis* showed 98.4% mortality against *Culex quinquefasciatus* at 100 ppm concentration and LC_{50} and LC_{90} value were 19.26 ppm and 84.84 ppm respectively after 24 h of exposure period (Arivoli et al., 2015). Dimetry et al., (2007) documented that *C. colocynthis* formulations are effective biopesticides against stored grain pest like adults of *Callosobruchus maculatus.* Similarly, aqueous extract from various parts of this plant extensively reduced the population of *Rhopalosiphum padi* (Asiry, 2015). The ethanolic extract from *C. colocynthis* exhibited the insecticidal effect against *Aphis craccivora* and this insecticidal potential is due to the presence of alkaloids, glycosides and saponins (Torkey et al., 2009). It has antifeedants, deterrent, growth-regulating and infertility properties against insects (Seenivasan et al., 2004) and familiar in the traditional medicine and used as purgative, anti-inflammatory, cathartic and pain relieving properties (Mehrzadi et al., 2016; Memon et al., 2003; Shi et al., 2014)

Cannabis is another paramount annual weedy and dioceous plant belongs to family Cannabaceae. Historically, it appears to have been cultivated in Northern China since 4000 BC (Mabberley, 2008). *Cannabis* is used as a pest repellent and has been grown as a companion crop to kill several insects, fungi, weeds and nematodes. Its extracts have the ability to repel or kill insects, mites, and microorganisms and can be used as allelochemicals. *Cannabis* leaves have great potential against pests (McPartland, 1997) and these insecticidal properties of plants can be found in their secondary metabolites. For example, crude extract from *Chenopodium ficifolium* and *Jatropha curcas* exhibits aphicidal activity, and alkaloids from *Corydalis turtschaninovii* tubers and *Macleaya cordata* seeds harbour insecticidal potential (Abdoul Habou et al., 2011; Le Dang et al., 2010; Park et al., 2011; Rashid et al., 2013). The essential oil of *Cannabis* has demonstrated antimicrobial activity (Nissen et al., 2010; Novak et al., 2001) while, essential oil from Nepalese origin of *C. sativa* was evaluated for cytotoxic, larvicidal and insecticidal activity, and was found as relatively non-toxic (Satyal and Setzer, 2014).

The main bioactive components i.e. flavonoids, alkaloids, terpenoids and phenols from plant origin show strong insecticidal activity. Benelli et al. (2018) reported that essential oil obtained from fresh inflorescence of *C. sativa* dominated by monoterpenes and sesquiterpenes showed strong toxic effects against *M. domestica* at the rate of 43.3 μg/adult and *M. persicae* with LC_{50} of 3.5 ml/l, moderately toxic against *S. littoralis* larvae at the rate of 152.3 μg/larva while least toxic against *C. quinquefasciatus* larvae with LC_{50} of 252.5 ml/l. It contains pesticidal properties however, tetrahydrocannabinol (THC) is the prime constituent and sixty six other cannabinoids are reported including cannabidiol (CBD) and cannabinol (CBN) showing different effects (Fusar-Poli et al., 2009). Moreover, Pellati et al. (2018) analyzed the bioactive components from inflorescences of *C. sativa* (hemp) by means of innovative HPLC and GC methods coupled with GC-MS and GC-FID. Analysis showed the abundance of cannabidiol (CBD) and Cannabidiolic acid (CBDA) while, β-caryophyllene and β-myrcene were the major terpenes.

Plants are also a source of cheap antioxidant substitutes; they have the ability to scavenge free radicals, avoid the reaction transmission and hence prevent humans from their damage (Moure et al., 2001). Phenolic compounds, flavonoids and terpenes collected from natural plant resources have potential free radical scavenger activity (Mathew and Abraham, 2006) and are omnipresent groups of plant secondary metabolites (Singh et al., 2007).

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The phytochemical extraction mechanism is important for obtaining better extract yield both qualitatively and quantitatively and scientists have studied the importance of *C. colocynthis* and *C. sativa* as a medicinal, insecticidal and antibacterial agent, but existing knowledge and research presented by different authors is limited to use of few solvents for extraction, extract properties as well as qualitative, quantitative analysis and antioxidants properties are not properly investigated in such a comprehensive way. Although, selected plants belong to different families and also harvested from different locations but it is worth noted that selected plants are potential source of phytochemicals and antioxidants responsible for insecticidal activities. Thus, at present extensive studies have been conducted on qualitative and quantitative extract estimations to identify different phytochemicals responsible for insecticidal potential and to determine the best extraction solvent with maximum phenolic and flavonoid contents and antioxidant activities using seven different solvents from *C. sativa* (leaves) and *C. colocynthis* (leaves and roots) collected from China and Pakistan respectively over the years 2017-2018.

Materials and methods

Plant material collection

Citrullus colocynthis (Colocynth), locally known as Tumba, and *Cannabis indica* formerly called as *Cannabis sativa* sub-species *indica* (Hemp) were the study plants. Young leaves and roots of Colocynthis were collected from (Bahawalnagar) a desert area of Punjab Province, Pakistan from March-April 2018 (*Fig. 1a* and *1b*), while hemp leaves were collected from different locations of Shenyang, Liaoning Province, China from August-September 2017 (*Fig. 1c*). In total ten samples of each plant were collected belongs to same area and same species. Collected samples of colocynthis and hemp were then mixed together separately to obtain bulk volume of each plant. Botanical identification of colocynthis as *C. colocynthis* was performed at Department of Botany Pir Mehar Ali Shah Arid Agriculture University Rawalpindi (PMAS, AAUR) Pakistan, while hemp was identified as *C. sativa* by Professor Ji Mingshan, Department of Pesticides Science, College of Plant Protection, Shenyang Agriculture University, Shenyang Liaoning China.

Sample preparation

Leaves and roots were washed, dried under shade at room temperature, crushed into a fine powder by using an electric blender, sieved through 100 µm mesh and finally 1 kg of each plant sample was obtained and stored in airtight glass jars at 4°C.

Solvent extraction

Different solvents were selected with varying polarity level to extract crude material by cold extraction method which was simple, easy and safe for elution of bioactive compounds along with pigments (Handa et al., 2008). For each solvent extraction of each bulk plant material, 100 g of powdered sample was taken in a 1000 ml conical flask separately and extracted with 400 ml of respective solvent (Mondal, 2015). Extraction was performed at room temperature for 72 h (Mojab et al., 2010; Senguttuvan et al., 2014). The extraction process was repeated thrice to ensure that all polar and non-polar materials were eluted completely (Waiganjo et al., 2013). Filtered the contents, and

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concentrated to reduce the volume on a rotary evaporator (Buchi Switzerland R-210). The concentrated filtrate was dried in a fume cupboard at 25°C. Solvent-free extract was placed in a stoppered glass bottle and stored at 4°C for further use. The physical properties of each extract (colour, stickiness and appearance) were recorded visually.

Figure 1. a *C. colocynthis plant.* ***b*** *C. colocynthis (roots).* ***c*** *C. sativa plant*

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Aqueous extraction

A total of 100 g powdered plant samples was extracted at room temperature with 400 ml of distilled water for 72 h. The rest of the procedure was same as for solvent extraction.

Calculation of extract yield (g)

The extract yield was calculated for each solvent from the respective plant material and stored at 4°C for further studies.

Model validations

The relationship was used to develop the model for yield and for the comparison of coefficient of determination (R^2) values with root mean square error (RMSE). The coefficient of determination R^2 and RMSE represent the standard deviation of the residuals (prediction errors). Residuals are the measures that how the values are far from the regression lines where the data points were. This measure also told us how condensed the data were around the line of best fitness and model validity (Hossain et al., 2017). Performance of the model in the crude extract yield was evaluated by using RMSE. In the experiment with observations 'n', RMSE was calculated by identity *Equation 1* (Debaeke et al., 1997).

$$\mathbf{RMSE} = \sqrt{1/n \times \sum_{j=1}^{n} ((Yield_{mes} - Yield_{sim})^2} \quad \text{(Eq.1)}$$

Qualitative phytochemical analysis

Phytochemical screening tests were performed to evaluate the presence or absence of phytoconstituents in *C. colocynthis* and *C. sativa* extracts using standard methods.

Test for alkaloids

For the estimation of alkaloids, 0.5 ml of each solvent extract was allowed to dry and 2 ml of 2% hydrochloric acid (HCl) was added to the residue in the test tube for 15 min in a water bath at 100°C. After cooling, the mixture was filtered and divided into two equal portions. A few drops of Mayer's reagent were added to one portion, while a few drops of Dragendoff's reagent were added to the other portion. Turbidity or the presence of a yellow precipitate confirm the presence of alkaloids (Siddiqui and Ali, 1997).

Test for glycosides

The presence of glycosides was evaluated by adding 2 ml distilled water and 2 ml 5% ferric chloride ($FeCl_3$) into 2 ml extract. The mixture was heated for 15 min in a water bath and then allowed to cool. Next, 1 ml benzene was added to the mixture and subjected to settle for 1 min after shaking. Next, 4 to 5 drops of concentrated ammonia (NH_3) was added. A pink or red colour indicates the presence of glycosides (Siddiqui and Ali, 1997; Siddiqui et al., 2009; Sofowora, 1993).

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Test for terpenoids and steroids

The presence of terpenoids and steroids was determined by a previously described method (Kantamreddi and Lakshmi; Siddiqui and Ali, 1997) with slight modifications. Briefly, 0.5 g of solvent-free extract was added in 2 ml chloroform and then filtered. The filtrate was placed on ice; addition of 2 ml of acetic acid and then a few drops of concentrated sulfuric acid (H_2SO_4) was carefully applied to the inner sides of the test tubes. The emergence of a pink or pinkish brown colour/ring indicates the terpenoids existence, while a blue or bluish green colour for steroids presence and the combination of pink and blue/bluish green colours confirmed the presence of both terpenoids and steroids.

Test for flavonoids and flavones

Two millilitres of diluted sodium hydroxide (NaOH) was added to the 3 ml extract solution, which turned the solution into a yellow colour. The solution was treated with 1 ml 5N hydrochloric acid (HCl), which turned the solution colourless, indicate flavonoids, and an orange colour indicate flavones (Siddiqui and Ali, 1997; Siddiqui et al., 2009; Sofowora, 1993).

Test for tannins

For the estimation of tannins, 1 ml distilled water and ferric chloride ($FeCl_3$) 1-2 drops were added to 0.5 ml plant extract. The appearance of a blue and green/black colour indicates gallic and catecholic tannin, respectively (Iyengar, 1995).

Test for phenols

To detect phenols, 1 ml of extract solution was added to 2 ml distilled water and a few drops of 10% ferric chloride ($FeCl_3$). The appearance of a green or blue colour is the indication of phenols presence. Next, 0.50 g of plant extract was added and allowed to dissolve in distilled water and then 3 ml of 10% lead acetate was added. The presence of phenolic compounds was confirmed by the appearance of white precipitation (Trease et al., 2003).

Test for saponins

One gram of solvent-free extract was diluted with 20 ml of distilled water and shacked vigorously. The formation of a foam layer (1 cm) indicates the presence of saponins (Siddiqui and Ali, 1997).

Quantitative phytochemical analysis

Determination of total phenolic content (TPC)

The total phenols present in the crude extract of *C. colocynthis* and *C. sativa* was calculated by the Folin-Ciocalteu reagent method, with slight modifications. Briefly, 1 ml plant extract (1 $mgml^{-1}$ of solvent) was added to 2.5 ml of 10% Folin-Ciocalteu reagent (1:1) and 2 ml of 2% sodium carbonate (Na_2CO_3). The consequential mixture was allowed to stand for incubation for 15 min at room temperature in the dark. The solution was then transferred to a 96-well ELISA plate to measure the absorbance at 765 nm using an absorbance microplate reader (SpectraMax 190, manufactured in

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China; designed in USA). All test calculations were carried out in triplicate. A standard calibration curve was constructed using gallic acid (1 $mgml^{-1}$). The results were calculated from the standard curve obtained by using different concentrations of gallic acid (1, 0.50, 0.25, 0.10, 0.05, 0.02, 0.01 and 0 $mgml^{-1}$) and are expressed as gallic acid equivalent (GAE) mgg^{-1} of extracted compounds (Aiyegoro and Okoh, 2010).

Determination of total flavonoid content (TFC)

The amount of total flavonoids in the crude extract of *C. colocynthis* and *C. sativa* was estimated by a colourimetric method using aluminium trichloride ($AlCl_3$) and sodium hydroxide (NaOH). Briefly, 1 ml of extract (1 $mgml^{-1}$ of solvent) was added to 3 ml methanol, 0.2 ml 10% aluminium chloride, 0.2 ml 1M potassium acetate (CH_3COOK) and 5.6 ml distilled water. The resulting mixture was incubated at room temperature in the dark for 30 min. The assay was performed at 420 nm in a 96-well ELISA plate. Quercetin was used as the standard (1 $mgml^{-1}$) and a standard curve was obtained using different concentrations of quercetin (1, 0.50, 0.25, 0.10, 0.05, 0.02, 0.01 and 0 $mgml^{-1}$). Results are expressed as quercetin equivalent (QE) mgg^{-1} of extracted compounds (Aiyegoro and Okoh, 2010). All test determinations were carried out in triplicate.

Determination of DPPH radical scavenging activity

The antioxidant activities of crude extracts of *C. colocynthis* and *C. sativa* were measured on the basis of the scavenging activities of the stable 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical (Yu et al., 2003). Briefly, 0.5 ml of each respective extract was added to a sample cavity containing 3.5 ml of freshly prepared methanol solution of DPPH (0.004 $g100ml^{-1}$). The mixture was subjected to incubation for 30 min at room temperature in the darkness and absorbance was calculated at 517 nm. The percent inhibition of DPPH (I %) was calculated from the decrease of absorbance by *Equation 2*. A lower absorbance value represents higher free radical scavenging activity (Zhao et al., 2008).

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad \text{(Eq.2)}$$

Whereas A_{blank} = absorbance of control; A_{sample} = absorbance of samples.

Statistical analysis

Data were analysed statistically by one-way analysis of variance (ANOVA) in Duncan's multiple range test (DMRT) and values are represented as the mean±standard deviation, with P = 0.05 indicating a significant difference. Analyses were conducted by using SPSS 13.0 (Inc.) software while graph was constructed by sigma Plot software.

Results

Potential solvent for extraction

Extraction was performed on *C. colocynthis* leaves and roots and on *C. sativa* leaves using different solvents and distilled water. Extract yields (g) was calculated and

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presented in *Figure 2* while extract properties were described in *Table 1*. *Figure 2* and *Table 1* showed that using distilled water, *C. sativa* leaves produced a significant yield (11.688 g), with the physical properties of dark brown colour, non sticky and dry solid, followed by methanol (9.412 g), black shiny, more sticky, and ethanol (6.296 g), blackish and shiny.

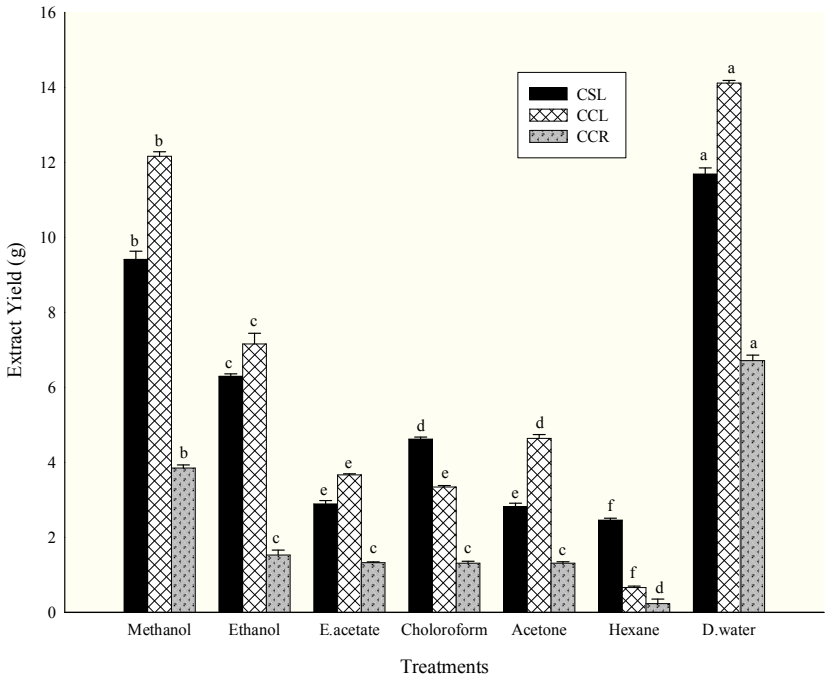

Figure 2. *Extract yield (g) of C. sativa leaves (CSL) and C. colocynthis leaves (CCL) and roots (CCR) by using different solvents. Highest yield produced by D. water and methanol while lower yield produced by hexane from CCL, CSL and CCR respectively. Data corresponding to treatments labelled with different letters are significantly different (Duncan's multiple range test DMRT at P ≤ 0.05); the error bars represent standard of the means*

Table 1. *Properties of CSL, CCL and CCR crude extract*

Solvents extract	CSL			CCL			CCR		
	Colour	Stickiness	Appearance	Colour	Stickiness	Appearance	Colour	Stickiness	Appearance
Methanol	Black, shiny	More sticky	Slowly flowable	Greenish black, shiny	Sticky	Oily, flowable	Brown	Non-sticky	Semi solid, non-oily
Ethanol	Blackish, shiny	More sticky	Slowly flowable	Brownish black, shiny	Sticky	Oily, flowable	Yellowish brown	Non-sticky	Dry, waxy
E. acetate	Blackish	Sticky	Oily, flowable	Blackish	Sticky	Oily	Dark brown	Less sticky	Dry, non-oily
Chloroform	Brownish black	Less sticky	Less oily, non-flowable	Black, Shiny	Less sticky	Less oily, non-flowable	Brown	Less sticky	Semi-solid, non-flowable
Acetone	Brownish black	sticky	Oily, flowable	Greenish black	Sticky	Oily	Yellow	Non-sticky	Dry, Non-flowable
Hexane	Yellowish	Less sticky	Non-oily, non flowable	Yellowish brown	Less sticky	Non-oily, non flowable	Light yellow	Non-sticky	Semi-solid, non-flowable
Distilled water	Dark brown	Non-sticky	Dry solid	Dark brown	Less sticky	Less oily	Brown	Less sticky	Dry, non-flowable

CSL (*C. sativa* leaves), CCL (*C. colocynthis* leaves), CCR (*C. colocynthis* roots)

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C. colocynthis leaves produced significant yield (14.115 g) using distilled water, with the physical properties of dark brown colour, less sticky followed by methanol (12.161 g), greenish shiny black and sticky, and ethanol (7.158 g), brownish black shiny and sticky.

C. colocynthis roots recorded a significantly high yield by distilled water (6.712 g), with the physical properties of dark brown colour and less sticky, followed by methanol (3.848 g), with the physical properties of greenish black, oily and ethanol (1.528 g), yellowish brown and waxy. However, the yields recorded by ethyl acetate (1.324 g), chloroform and acetone (1.312 g) were non significant ($P > 0.05$) to each other but differed significantly compared with all other treatments.

Model validation

The model validation performance for extract yield in response to *C. sativa* leaves and *C. colocynthis* leaves and roots is listed in *Table 2*. The *C. sativa* leaves extract exerted significant ($P < 0.001$) effects on the aqueous extract compared to all other treatments. The extract yield revealed that R^2 values of *C. sativa* using methanol, ethanol, ethyl acetate, chloroform, acetone, hexane and distilled water were 0.99, 0.93, 0.92, 0.87, 0.87, 0.93 and 0.82 respectively showed better performances compared to RMSE. Data on the extract yield for *colocynthis* leaves were statistically analysed and recorded as significant ($P < 0.05$) when obtained by methanol, with R^2 values of 0.99, 0.80, 0.98, 0.99, 0.81, 0.99 and 0.91. These extract yields for *C. colocynthis* roots were highly significant ($P < 0.01$) when obtained by distilled water and methanol compared to all other treatments. However, R^2 values of *C. colocynthis* roots 0.89, 0.84, 0.95, 0.94, 0.99, 0.94 and 0.92 showed the best curvature and best fit to the obtained data.

Table 2. *Regression model for phytochemical screening of cumulative extract yield*

Solvent extract	CSL		CCL		CCR	
	R^2	*RMSE*	R^2	*RMSE*	R^2	*RMSE*
Methanol	0.99	0.045266	0.99	0.0041231	0.89	0.0360786
Ethanol	0.93	0.588661	0.79	0.2680105	0.84	0.0989949
Ethyl acetate	0.92	0.075688	0.97	0.0245221	0.95	0.0070711
Chloroform	0.87	0.009556	0.99	0.0311127	0.94	0.0141421
Acetone	0.87	0.024042	0.81	0.1209201	0.99	0.0023804
Hexane	0.93	0.024338	0.99	0.0028284	0.94	0.1075546
Distilled water	0.82	0.308299	0.91	0.0268886	0.92	0.0287171

R^2 (Correlation coefficient), RMSE (Root mean square error), CSL (*C. sativa* leaves); CCL (*C. colocynthis* leaves); CCR (*C. colocynthis* roots)

Qualitative phytochemical analysis

Tests for phytochemical screening were conducted to determine the presence of bioactive compounds, namely, alkaloids, glycosides, terpenoids, flavonoids, flavones, steroids, tannins, phenols and saponins from *C. colocynthis* and *C. sativa* (*Tables 3, 4* and *5*). However the confirmation of a bioactive compound was referred as high presence (++) on strong resemblance; moderate presence (+) on medium or weak and (-) with no resemblance, appearance or change of colour of the reactive solution. The detection of alkaloids in the solvent extract was confirmed by turbidity or the emergence of a yellow

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precipitate. The glycosides presence was confirmed by the appearance of a red or pink colouration in the reaction mixture. Positive results for terpenoids were confirmed by *C. colocynthis* roots, with the appearance of a pink or pinkish brown colour. Flavonoids and flavones were recorded by the transformation of the mixture from yellow to colourless and orange, respectively. The presence of tannins was confirmed by the appearance of a blue colour for gallic tannin and green/black for catecholic tannin. Blue or green colour in the reaction mixture indicated the presence of phenols while, foaming character in extract solution is important for the confirmation of saponins.

Phytochemical analysis indicated a high presence of steroids in chloroform and hexane extracts and a moderate presence in most other solvent extracts from *C. sativa* leaves except for distilled water extract (*Table 3*). Terpenoids were absent in all extracts. Alkaloids were absent in ethyl acetate, chloroform and distilled water extracts, while glycosides were present in all of these extracts except for hexane. Moreover, flavonoids were highly present in methanol and ethanol but moderately present in acetone and hexane. Flavones were present in acetone, as well as hexane and distilled water extracts. Saponins were present in chloroform, hexane and Distilled water extracts. However, gallic tannins were absent in all extracts, while catecholic tannins were absent in acetone, hexane and distilled water extracts. Phytochemical screening showed that phenols were present in methanol, ethyl acetate, ethanol and distilled water extracts.

Table 3. *Qualitative phytochemical screening of C. sativa leaves crude extract*

Solvents extract	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Flavones	Tannins		Phenols	Saponins
							Catecholic	Gallic		
Methanol	+	-	-	+	++	-	+	-	+	-
Ethanol	+	-	-	+	++	-	+	-	+	-
Ethyl acetate	-	+	-	+	-	-	+	-	+	-
Chloroform	-	+	-	++	-	-	-	-	-	+
Acetone	+	-	-	+	+	+	+	-	-	-
Hexane	+	+	-	++	+	+	-	-	-	+
Distilled water	-	-	-	-	-	+	-	-	+	+

++high presence, +moderate presence, -absence

Table 4. *Qualitative phytochemical screening of C. colocynthis leaves crude extract*

Solvents extract	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Flavones	Tannins		Phenols	Saponins
							Catecholic	Gallic		
Methanol	+	-	-	+	++	-	+	-	++	-
Ethanol	-	+	-	+	++	-	+	-	+	-
Ethyl acetate	-	+	-	+	++	+	-	++	++	-
Chloroform	-	++	-	+	+	+	-	-	-	-
Acetone	-	-	-	+	-	-	+	-	+	-
Hexane	-	++	-	+	+	+	-	-	-	-
distilled water	-	-	-	-	-	-	-	-	+	+

++high presence, +moderate presence, -absence

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Table 5. *Qualitative phytochemical screening of C. colocynthis roots crude extract*

Solvents extract	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Flavones	Tannins		Phenols	Saponins
							Catecholic	Gallic		
Methanol	-	-	-	-	++	+	-	-	-	+
Ethanol	-	-	++	-	++	+	-	-	-	-
Ethyl Acetate	-	-	+	-	+	-	-	-	-	+
Chloroform	-	-	+	-	+	-	-	-	-	-
Acetone	+	-	+	-	+	-	-	-	-	-
Hexane	-	-	-	-	+	-	-	-	-	-
Distilled water	-	+	-	-	-	-	-	-	-	+

++high presence, +moderate presence, -absence

C. colocynthis leaves showed a moderate presence of phenols by methanol, ethanol, ethyl acetate, acetone and distilled water but an absence in other extracts. However, a high glycoside presence was recorded by chloroform and hexane, with a moderate presence by ethanol and ethyl acetate (*Table 4*). Alkaloids were absent in all extracts except for methanol. Terpenoids were absent in all extracts. A high presence of flavonoids was recorded in methanol, ethanol and ethyl acetate, while a moderate presence of flavonoids was recorded in chloroform and hexane extracts. Flavones were present in ethyl acetate, chloroform and hexane extracts. Steroids were present in all extracts except for distilled water. Tannins were present in methanol, ethanol, ethyl acetate and acetone extracts, while saponins were present only in distilled water extract.

As shown in (*Table 5*), the phytochemical analysis of *C. colocynthis* roots demonstrated the presence of glycosides and alkaloid in distilled water and acetone extract, respectively. Steroids, tannins and phenols were absent in all extracts. However, terpenoids showed a high presence in ethanol and a moderate presence in ethyl acetate, chloroform and acetone extracts but an absence in methanol and distilled water. Although the presence of flavonoids was recorded in all extracts except for distilled water extract, flavones were recorded by methanol and ethanol only. The presence of saponins was recorded by methanol, ethyl acetate and distilled water extract.

Quantitative analysis

Total phenolic content (TPC)

The total phenolic contents of various extracts from *C. colocynthis* leaves and roots and *C. sativa* leaves varied slightly. The maximum and minimum phenolic contents from *C. sativa* leaves were recorded by methanol and distilled water (36.42±1.905 and 29.98±0.56 mg gallic acid equivalent GAE/g, respectively). However, minimum phenols recorded by ethyl acetate and ethanol were (12.16±0.977 and 2.70±0.109 mg GAE/g) respectively while other solvents recorded negative phenols (*Table 6*). Methanol and distilled water extracts from *C. colocynthis* leaves showed a higher phenolic content of 37.69±0.35 and 37.25±0.83 mg GAE/g, respectively, followed by acetone, ethanol and ethyl acetate (24.08±1.78, 22.82±0.37 and 5.73±0.56 mg GAE/g, respectively). However, *C. colocynthis* roots showed negative results for phenols.

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Table 6. *Total phenolic content (TPC) from CSL, CCL and CCR*

Solvents extract	Total phenolic contents mg GAE/g		
	CSL	CCL	CCR
Methanol	36.42±1.905 [a]	37.69±0.35 [a]	-
Ethanol	2.70±0.109 [d]	22.82±0.37 [b]	-
Ethyl acetate	12.16±0.977 [c]	5.73±0.561 [c]	-
Chloroform	-	-	-
Acetone	-	24.08±1.78 [b]	-
Hexane	-	-	-
Distilled water	29.98±0.56 [b]	37.25±0.83 [a]	-

Results are presented as the mean values ± standard deviation. Same letters within a column indicate that mean values are not significantly different (P > 0.05) according to Duncan's multiple range test; CSL (*C. sativa* leaves); CCL (*C. colocynthis* leaves); CCR (*C. colocynthis* roots)

Total flavonoid content (TFC)

The total flavonoid content was high in the methanol and ethanol extracts of *C. sativa* leaves (59.03±1.31 and 56.00±1.85 mg quercetin equivalent QE/g, respectively), followed by hexane and acetone (17.35±0.43 and 12.08±0.62 mg QE/g, respectively) (*Table 7*). Thus, ethyl acetate extract from *C. colocynthis* leaves produced the highest flavonoid content (119.63 mg QE/g), followed by ethanol, methanol and hexane (54.84±0.90, 50.58±0.85 and 22.7±1.14 mg QE/g, respectively). However, the lowest flavonoid content was detected by chloroform (1.73±0.09 mg QE/g). The total flavonoid content of *C. colocynthis* roots by methanol and ethanol was the highest (43.32±0.33 and 42.34±0.23 mg QE/g, respectively), followed by ethyl acetate and acetone (28.93 and 8.86 mg QE/g, respectively). Chloroform and hexane extracts showed the lowest flavonoid content (2.23±0.18 and 1.23±0.09 mg QE/g, respectively).

Table 7. *Total flavonoids content (TFC) from CSL, CCL and CCR*

Solvents extract	Total flavonoid contents mg QE/g		
	CSL	CCL	CCR
Methanol	59.03±1.312 [a]	50.58±0.85 [c]	43.32±0.33 [a]
Ethanol	56.001±1.85 [b]	54.84±0.9 [b]	42.34±0.235 [b]
Ethyl acetate	-	119.63±0.31 [a]	28.93±0.19 [c]
Chloroform	-	1.7318±0.09 [e]	2.233±0.182 [e]
Acetone	12.086±0.62 [d]	-	8.863±0.154 [d]
Hexane	17.35±0.43 [c]	22.7±1.14 [d]	1.2343±0.094 [f]
Distilled water	-	-	-

Results are presented as the mean values ± standard deviation. Same letters within a column indicate that mean values are not significantly different (P > 0.05) according to Duncan's multiple range test; CSL (*C. sativa* leaves); CCL (*C. colocynthis* leaves); CCR (*C. colocynthis* roots)

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DPPH radical scavenging activity

DDPH is a stable and free radical which is easily dissolved in methanol showed characteristics colour absorption at 517 nm using spectrophotometer. Free radicals are scavenged by antioxidant molecules due to donation of hydrogen molecules and the colour of DPPH assay solution changed to light yellow colour causing reduction of absorbance. To determine free radical scavenging activity, the DPPH radicals are widely used. Data on the 1,1-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of free radical of *C. sativa* leaves and *C. colocynthis* leaves and roots are presented in (*Table 8*).

Table 8. *DPPH radical scavenging activity from CSL, CCL and CCR*

Solvents extract	DPPH Inhibition (%)		
	CSL	CCL	CCR
Methanol	49.5±0.7 [b]	30.65±1.07 [e]	23.63±0.68 [f]
Ethanol	54.1±0.2 [a]	36.52±0.62 [c]	33.83±0.351 [b]
Ethyl acetate	46±1 [c]	27.66±1.53 [f]	27.65±0.01 [e]
Chloroform	40.6±0.8 [d]	29.64±0.05 [e]	30.69±0.8 [c]
Acetone	55.57±1.2 [a]	33.5±0.51 [d]	30.97±0.98 [c]
Hexane	41.7±0.5 [d]	45.98±0.14 [a]	29.12±0.7 [d]
Distilled water	34.2±1.1 [e]	39.8±0.21 [b]	35.5±0.16 [a]

Results are presented as the mean values ± standard deviation. Same letters within a column indicate that mean values are not significantly different ($P > 0.05$) according to Duncan's multiple range test; CSL (*C. sativa* leaves); CCL (*C. colocynthis* leaves); CCR (*C. colocynthis* roots)

For *C. sativa* leaves, DPPH inhibition (%) by distilled water and acetone ranged from 34.2±1.10 to 55.57±1.20%. For *C. colocynthis* leaves, the DPPH inhibition (%) of chloroform and hexane extract ranged from 29.64±0.05 to 45.98±0.14%. However, the DPPH scavenging activity of *C. colocynthis* roots for methanol and distilled water ranged from 23.63±0.68 to 35.5±0.16%. It was also noted that all extracts of *C. sativa* and *C. colocynthis* showed varying levels of DPPH radical scavenging activity.

Discussion

The comparative extraction of crude extracts from *C. colocynthis* and *C. sativa* demonstrated that using methanol as an extraction solvent resulted in the maximum extract yield followed by ethanol, chloroform, ethyl acetate, acetone and hexane. However, using distilled water instead of organic solvent produced the maximum yield. Our results are consistent with (Awang et al., 2016; Markom et al., 2007; Pin et al. 2009) who reported that the highest extract yield was obtained by using water for extraction from *M. malabathricum* leaves, *Phyllanthus niruri* and *Piper betel*. Among the polarity-based solvents, methanol produced the maximum yield, followed by ethanol. Similar results were demonstrated by (Dhawan and Gupta, 2017; Paulsamy and Jeeshna, 2011) that methanol produced the maximum extract yield from *Datura metel* and *H. radicata* leaves.

The extract yield revealed that R^2 relationship of *C. sativa* ranging from 0.82-0.99 for distilled water and methanol have strong positive correlation compared to RMSE 0.308-

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0.045 which showed better performance of model fitness. The trendline regression R^2 for *C. colocynthis* leaves were 0.99 for methanol, chloroform and hexane showed strong correlation while for *C. colocynthis* roots 0.99 by acetone recorded the best curvature and fitness of the model with recorded data.

The extraction of secondary metabolites highly depends on the extraction technique, solvent used and the chemical properties of the compounds. The analysis of *C. Sativa* leaf extracts showed the presence of alkaloids, glycosides, steroids, flavonoids, flavones, tannins, phenols and saponins and the absence of terpenoids. Relevant results were presented by Audu et al. (2014), who reported the presence of phytochemicals except for saponins and phenols in the leaves and roots of *C. sativa*. Various bioactive compounds such as flavonoids, alkaloids, glycosides, carbohydrates and essential oils have been documented previously from *C. colocynthis* by Wasylikowa and Van der Veen (2004). These isolated compounds like Flavonoids, isovitexin, isosaponarin and isoorientin 3'-*O*-methyl ether are revealed as significant antioxidants (Kumar et al., 2008). The results described by Najafi et al. (2010) on the phytochemical constituents from *C. colocynthis* leaves showed the presence of alkaloids, glycosides, tannins, flavonoids and saponins, consistent with our results. However, the results from our study are strongly correlated with Uma and Sekar (2014), who reported the presence of secondary metabolites in *C. colocynthis* leaves and roots. Singh (2010) reported the presence of bioactive compounds but no saponins or anthraquinones were reported from C. *colocynthis* roots.

C. colocynthis contains mostly flavonoids, while *Ziziphus spina-christi* contains the highest phenolic content (Tawfik et al., 2015). Phenols are biologically active compounds that are potent antioxidants with free radical scavenging behaviour. Haniyeh et al. (2016) reported that phenolic and flavonoid contents from the methanol extract of *C. Colocynthis* leaves were 79.78 and 45.46 μgml^{-1}, respectively. Similar findings were also observed herein, with phenolic and flavonoid contents of 37.69 and 50.58 mgg^{-1}, respectively. However, phenols were absent in all extracts of *C. colocynthis* roots. Moreover, Rizvi et al. (2018) reported the presence of polyphenol content from *C. colocynthis* leaves using different polarity solvent, methanol, ethyl acetate, chloroform, hexane and distilled water 74.34. 55.16, 56.32, 67.61 and 86.6 $mg100g^{-1}$ respectively which is relevant to our findings for phenol content from *C. colocynthis* leaves. Maximum phenol and flavonoids content recorded by methanol from *C. sativa* 36.42 and 59.03 mgg^{-1} are consistent with Abd-Alla and Haggag (2013) who reported total phenol and flavonoid contents of 9.62 mgg^{-1} and 1.9 mgg^{-1}, respectively, with 14.5% antioxidant activity from the leaf extract of *C. sativa*.

The results from different studies have revealed that phenols, flavonoid content and antioxidant properties differ in different parts of *C. colocynthis*. Plants are an active source of valuable chemicals and other bioactive compounds that contain pesticidal activity against numerous insect pests (Koul and Walia, 2009). Salama and Al Rabiah (2015) reported the presence of phenol, flavonoid and antioxidant activities from twenty medicinal plants, with maximum phenol and flavonoid contents of 224.49 mg/100 g and 126.24 $mg100g^{-1}$, respectively, from *C. colocynthis* (seeds). However, the inhibition percent in the ethanol extracts of roots and leaves was 56.8-67.2% and 5.97-6.42 μgml^{-1}, respectively. Among the ethanol extracts, leaves had the highest free radical inhibition activity in *C. colocynthis* (IC_{50} = 2.97, 67.2%) (Hussain et al., 2013). Our findings also demonstrated that inhibition (%) in the ethanolic extract from leaves and roots was 36.52 and 33.83%, respectively. However, there are variations in antioxidants contained

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by *C. sativa* and *C. colocynthis* among different solvents extract. Additionally, Eddouks et al. (2002) reported that methanolic extract of *C. colocynthis* seed exhibited highest inhibition 79.4 and 72.4% by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Rathanavel and Arasu (2014) documented that Colocynth contains several phytoconstituents, such as flavonoids, alkaloids, glycosides, tannins saponins, carbohydrates and essential oils responsible for insecticidal activities.

Results from phytochemical analysis of *C. sativa* and *C. colocynthis* are characterized by the presence of various phytochemicals along with phenols, flavonoids content and antioxidant activity. However, data on previous studies for extraction and comparing the extract yield using different solvent, qualitative and quantitative analysis, antioxidant activity and use of bioactive compounds as alternative of synthetic chemicals from these plants is limited. Also synthetic agents in present era liberate maximum residues in environment and naturally grown population which is burning issue in current agro-ecosystem. So the study was conducted to examine extract yields using seven different solvents by solvent extraction method, to observe physical properties of extract, scrutinize bioactive compounds and to quantify total phenol, flavonoids contents and to asses' antioxidant activities by DPPH radical scavenging from crude extracts of study plants. Also solvents extract from *C. colocynthis* roots, yield calculation and physical properties were studied for the first time in such a comprehensive way.

Conclusion

The results demonstrated that crude extracts obtained by using different solvents from *C. colocynthis* and *C. sativa* possess biological compounds, such as terpenoids, glycosides, alkaloids, flavonoids, flavones, steroids, tannins, phenols and saponins. Owing to extraction, distilled water afforded the maximum yield followed by methanol with high phenol and flavonoid content. Hence, the studied plants are a rich source of phytocompounds, conferring them interesting antioxidant activity and supporting their use as potential insecticidal agents which are safe for environment and biodiversity. However, comprehensive research is needed to identify additional solvents and techniques for the extraction, isolation, purification and identification of active ingredient responsible for insecticidal activities.

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