Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in-vitro antioxidant activity evaluation of Cadaba rotundifolia Forssk leaf extracts

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Abstract: Leaves of Cadaba rotundifolia Forssk have widely been used by the community of Eastern Ethiopia as traditional phytomedicine against various diseases. The focus of this study was to screen major phytochemical classes and determine their contents, and evaluate in-vitro antioxidant activities of different solvent extracts of C. rotundifolia leaf. The total content of phenolic (TPC), flavonoid (TFC) and tannin (TTC), and in-vitro antioxidant activity (using DPPH and ferric reducing power assays) of four extracts (pure ethanol, pure methanol, 80% methanol and 80% ethanol) were determined using spectrophotometric method. The obtained data revealed that 80% ethanol extract scored the highest extraction yield (16.22%) followed by 80% aqueous methanol (12.90%), methanol (12.87%) and ethanol (12.86%); whereas the lowest crude extract percentage yield was recorded by petroleum ether (1.11%) followed by ethyl acetate (1.42%) and...
dichloromethane (1.65%). Flavonoids, phenols, alkaloids, saponins, carbohydrates and tannins were positively screened especially in the alcoholic and their corresponding aqueous extracts. The present finding also showed the presence of appreciable amounts of total phenolic (8.04 ± 1.04–10.46 ± 1.25) mgGAE/gDCE (gallic acid equivalents per gram of dried crude extracts), flavonoid (0.38 ± 0.05–0.51 ± 0.03) and tannin (0.75 ± 0.03–1.05 ± 0.03) mgCE/gDCE (catechin equivalent per gram of dried crude extracts). The 80% methanol extract exhibited the highest phenolic (10.46 ± 1.25 mgGA/gDCE) and flavonoid (0.51 ± 0.03 mgCE/gDCE) contents; whereas the highest total tannin content (0.92 ± 0.03 mgCE/gDCE) was recorded in aqueous (80%) ethanol extract. The aqueous ethanol and methanol extracts showed the highest scavenging activity against DPPH-free radical and ferric reducing antioxidant power (FRAP) than the non-aqueous parts; which is also correlated with the obtained total contents of phenolic, flavonoid and tannin expected to have antioxidant potency. In the immediate future, it is therefore very important to note that the hydro-alcoholic solvents should be prioritized while conducting any phyto-pharmacological studies on the C. rotundifolia leaves.

**Subjects:** Food Additives & Ingredients; Food Chemistry; Food Engineering

**Keywords:** Cadaba rotundifolia Forssk; DPPH assay; ferric reducing power assay; phytochemical screening; phytochemical contents

1. **Introduction**

This day, the chemical identity of reactive oxidative species and their devastative effect on the biological makeup of living cells have well been known. These restless and unstable reactive species destroy the major macromolecules of cells (such as proteins, lipids, enzymes and nucleic acids) thereby leading to the conversion of the functional cell to the malfunctioning one (Baba & Malik, 2015; Khatoon et al., 2013). Such malfunctioning cell causing oxidative stresses lead to various diseases like cancer, cardiovascular diseases, arteriosclerosis, neural disorders, Parkinson’s disease, Alzheimer's disease, ageing, hypertension, inflammations, diabetes mellitus and chronic fatigue syndrome (Rebaya et al., 2015). Now, the question is how these reactive free radical species can be defended by the living cells? Thus far, researchers have spent their precious efforts and time on searching effective, safe and sustainable alternatives to tackle the causative effect of the reactive species. Of those alternatives, the natural sources originated antioxidants have been found as the champion one. Plants are in the front line as well-known homes of various natural antioxidants; which currently have been gained serious attentions (Khatoon et al., 2013; Lefahal et al., 2018; Truong et al., 2019). These medicinal plant based natural antioxidants have played tremendous roles on fighting free radicals via the chemistry of providing an electron and/or hydrogen and making the restless oxidative species stable (Rebaya et al., 2015). It is very apparent that the credit of biological activities, including antioxidants of medicinal plants has been given to the phytochemical classes like polyphenols, saponins, flavonoids, carotenoids, vitamin E and alkaloids they harbour in (Upadhya et al., 2015). Phenolic compounds and flavonoids are among the well-known phytochemical classes, which scavenge free radicals by acting as reducing agents, donating hydrogen-free radical, quenching singlet oxygen and chelating metals (Chigayo et al., 2016; Iloki-Assanga et al., 2015).

*Cadaba rotundifolia* Forssk, an evergreen shrub, is classified under the family Capparaceae and genus *Cadaba*. *C. rotundifolia* contains leaves, which are densely sparsely with swollen- based hairs (*Figure 1*). The genus *Cadaba* also includes other 30 species which are distributed from Northeast Africa to South west Arabia (Karemy, 2001). In Ethiopia, *C. rotundifolia* is widely distributed in desert plain situated mainly within the Rift Valley lies in the Eastern and North Eastern part
Different parts of *C. rotundifolia* have been reported for their different ethnomedicinal uses. For example, the leaves of the plant have been applied to treat ailments such as cough, stomach ulcer and cancer in Yemene (Al-Fatimi, 2019) and as antibiotics in Rwanda (Hassan-Abdallah et al., 2013). In Sudan also, the roots and leaves of *C. rotundifolia* are traditionally used to treat tumors and abscesses (Graham et al., 2000). In the Eastern part of Ethiopia, the leaves of *C. rotundifolia* in combination with *Withania somnifera* are traditionally used to treat extended flow of menstruation/Menometrorrhagia (Belayneh & Busso, 2014; Hassan-Abdallah et al., 2013) external injury, wounds, skin infection and diarrhea (Teklehaymanot & Giday, 2010) and the aerial parts (leaves and stems) are known for treating arthritis, eye sickness, tonsillitis, flue, retained placenta, external parasite, brucellosis, bloating and bovine TB (Teklehaymanot, 2017). Moreover, ethanol extracts of the leaves also possess anti-malarial activity (Giday & Teklehaymanot, 2013). Regarding the nutritional value, *C. rotundifolia* has been reported for its high protein content (Belayneh et al., 2017). Thus far, Al Hamoud et al. (2019), reported a chemistry study about the chemical constituents of *C. rotundifolia*, the ethanolic root extract of *C. rotundifolia* resulted a quaternary alkaloid (3-hydroxystachydrine); and the aerial parts provided flavonoid glycosides and other flavonoids constituents. However, no studies were reported so far on the qualitative and quantitative investigation of major phyto-compounds, and *in-vitro* antioxidant activity of *C. rotundifolia* leaf extracts. Conducting an experimental investigation on the major phytochemical families and anti-oxidative potency of various extracts of *C. rotundifolia* leaf is therefore urgently needed. The present study focused on the qualitative screening of major class of compounds, quantitative analysis of total phenolics, flavonoids and tannins contents; and *in-vitro* antioxidant activities of various extracts prepared from *C. rotundifolia* leaf.

2. Materials and methods

2.1. Plant material collection
Leaves of *Cadabarotundifolia* were collected in January 2020 from Harla and Denggo mountains (9°27′ and 9°39′N latitude and 41°38′ and 42°20′E longitude) found in the Eastern part of Ethiopia. The
taxonomical name of the plant species was confirmed using the flora data/document of Haramaya University, Ethiopia and a voucher specimen was deposited at the University’s herbarium. The freshly collected leaves were then properly washed with tap water and rinsed with distilled water followed by air-drying under a shed at room temperature for a month. The dried leaves were ground into fine powder using an electrical grinder. The powdered leaf sample was then stored in a dark airtight glass container at 4°C refrigerator until extraction was commenced.

2.2. Chemicals and reagents
Ethanol (99.5%) and petroleum ether (40–60°C) were obtained from Carlo Erba reagents (France). Methanol (99%), dichloromethane (99%) and hydrochloric acid (37%) were purchased from Loba Chemie Pvt Ltd (India) while ethyl acetate from Sisco Research Laboratories (India) and DPPH (2, 2-Diphenyl picrylhydrazyl) from Alfa Aeser (Germany). Gallic acid and catechin reagents were bought from P5 Park (Northampton, UK). Ascorbic acid- (L), potassium ferricyanide and sodium nitrite were purchased from AnalRBDH (England). Aluminium chloride, trichloroacetic acid, sodium hydroxide and ferric chloride were procured from Blulux (India). Folin-Ciocalteureagent, sodium hydrogen carbonate, and vanillin were bought from Sigma-Aldrich (Sternheim, Germany). All the chemicals used, including the solvents were of analytical grade.

2.3. Preparation of plant extracts
Finely powdered leaves of Cadaba rotundifolia (40.00 g) was extracted with seven solvent systems (300 ml each), namely, petroleum ether, dichloromethane, ethyl acetate, 100% ethanol, 80% aqueous ethanol, 100% methanol and 80% aqueous ethanol, separately by placing on an orbital shaker at room temperature for 48 h. Each extracts were then filtrated using Whatman No.1 filter paper (Whatman International Ltd, England) and filtrates were concentrated using a rotary evaporator (Rotary vacuum, Jainsons, India) under reduced temperature and pressure; and the hydroalcoholic extracts were further dried over water bath at 90°C. Crude extracts were then stored in a refrigerator at 4°C for further analyses. The percentage of extraction yield (%) was determined using the following formula:

\[
\text{Extraction yield (\%) = \frac{\text{Weight of crude extract}}{\text{Weight of powdered sample}} \times 100}
\]

2.4. Phyto-chemical profiling test
Each crude extract was tested for the confirmation of the absence/or presence of some major classes of phytochemicals following the standard qualitative procedures described by Paterson (1999) with a slight modification.

2.4.1. Tests for flavonoids
Alkaline reagent test: 2 ml of 2.0% NaOH was mixed with each plant crude extract. An intense yellow colour was formed which turned colourless on the addition of two drops of diluted acid which indicated the presence of flavonoids.

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate also confirmed the positive test of flavonoids.

2.4.2. Test for alkaloids
The presence of alkaloids was tested using two reagents, Mayer’s and Wagner’s reagents. A few drops of the solution were added to the extract solution (0.5 ml). A reddish-brown turbidity or precipitate demonstrated the positive alkaloids.

2.4.3. Test for tannins (Ferric chloride test)
To the plant extracts solution (0.5 ml), few drops of 5% ferric chloride were added. Black or blue-green colouration or precipitate was taken as evidence for the presence of tannins.
2.4.4. Test for saponins
Few drops of NaHCO3 were added to the plant extract solution (0.5 ml) and shaken vigorously to froth and then allowed to stand for 15–20 min. A height of persistent foam greater than 1 cm indicated the presence of saponins.

2.4.5. Test for steroids and terpenoids
Salkowski test: 2 ml of each plant extract was mixed with 2 ml of chloroform followed by the addition of concentrated H2SO4 (2 ml) by shaking well. A red colour produced in the lower chloroform layer indicated the presence of steroids.

2.4.6. Test for carbohydrates
Molisch test: Plant extracts (2 ml) were treated with a few drops of alcoholic alpha-naphthol. Concentrated sulphuric acid (0.5 ml) was then poured slowly along the sides of the test tube. The appearance of purple to violet colour ring at the junction indicated the presence of carbohydrate.

2.4.7. Detection of phenols
Ferric chloride Test: Extracts were treated with 3–4 drops of ferric chloride solution. Formation of bluish-black colour indicated that the presence of phenols.

2.5. Quantitative determination

2.5.1. Total phenolic content (TPC)
The total phenolic content (TPC) in four plant extracts, namely, 100% ethanol, 100% methanol, 80% ethanol and 80% methanol extracts, was determined following Folin-Ciocalteu method described by Truong et al. (2019) with minor modification. In brief, Folin-Ciocalteu reagent (5 ml, 10%) was added to each tested plant extract solution (1 ml, 1 mg/ml). After 5 min, Na2CO3 (4 ml, 7.5%) was added and the mixture was incubated at room temperature for 30 min. A set of standard solutions of gallic acid (10–100 μg/ml) were prepared in the same manner as described for the extracts. The absorbance of the extracts and standard solutions was measured against the reagent blank at 760 nm with a UV/Visible spectrophotometer (Cecil CE4001 UV/Vis, Cambridge, England). The total phenolic content in all tested extracts was determined from the calibration curve and expressed as milligram of gallic acid equivalent (mgGAE/gDCE) per gram of the dried crude extracts. The experiment was repeated thrice for all concentrations of the extracts and standards.

2.5.2. Total flavonoid content (TFC)
Total flavonoid content was determined using aluminium chloride colourimetric method (Anwar & Przybylski, 2012) with some modification. Briefly, each tested plant extract (1 ml, 1 mg/ml) was taken into 10 ml volumetric flask and distilled water (5 ml) and NaNO2 (0.3 ml, 5%) were added. After 5 min, AlCl3 solution (0.5 ml, 5%) was added to the mixture followed by the addition of 1 M NaOH (2 ml) after another 5 min and diluted to the mark with distilled water. After the solution was vigorously mixed, its absorbance was measured at 415 nm using a UV/Visible spectrophotometer. Furthermore, serial standard solutions of catechin (70, 60, 50, 40 30, 20 and 10 μg/ml) were prepared from 0.1 mg/ml stock solution using the same technique described for the extracts above. The absorbance of the extracts and standard solutions was measured against the reagent blank at 415 nm with a UV/Visible spectrophotometer (Cecil CE4001 UV/Vis, Cambridge, England). The total flavonoid content was determined from the calibration curve and expressed as milligram of catechin equivalent per gram of dried crude extracts (mgCE/gDCE). The determinations of total flavonoid content in the tested extracts and standards were analyzed in triplicates.

2.5.3. Total condensed tannin content
Total condensed tannin (proanthocyanidin) contents in all extracts was analyzed using the modified procedure stated by Medini et al. (2014) and Rebaya et al. (2015) with the standard reference,
catechin. In sum, vanillin (5 ml, 4% in methanol) and concentrated HCl (1.5 ml) were added to each tested plant extract solutions (1 ml, 1 mg/ml). In addition, serial standard solutions of catechin (60, 48, 36, 24, 30, 12 and 0 µg/ml) were prepared. After 20 min of incubation, the absorbance of both the extract and standard concentrations was measured at 500 nm against methanol as a blank and the total condensed tannin was expressed as mg equivalent of catechin per (mgCE/g DCE) dried crude extracts. All instrumental readings were conducted three times.

2.6. In-Vitro anti-oxidation activity evaluation of plant extracts
The in-vitro antioxidant activities of four extracts (100% ethanol, 100% methanol, 80% ethanol and 80% methanol) were investigated using two assays, DPPH free radical and Ferric Reducing Power (FRP).

2.6.1. DPPH assay
The free radical trapping activity of the extracts and the positive control, ascorbic acid was evaluated against the unstable radical DPPH by applying the method described by Khorasani Esmaeili et al. (2015) with little arrangements. Five different concentrations, namely, 25, 50, 100, 150 and 200 µg/ml of tested plant extracts were prepared from a stock solution (1 mg/ml). Then, freshly prepared DPPH solution (2 ml, 0.004% in methanol) was added to each concentration and incubated at room temperature for 30 min. The absorbance of each concentration was measured in triplicate at 517 nm using UV-visible spectrophotometer (Cecil CE4001 UV/VIS, Cambridge, England). The methanolic solution of DPPH was used as a negative control. The free radical scavenging activity percentage of evaluated plant extracts against DPPH free radical was calculated using the following equation formula:

\[ \text{DPPH scavening activity (\%)} = \frac{A_0 - A}{A_0} \times 100\% \]

Where \( A_0 \) and \( A \) are the absorbance of the negative control (0.004% w/v DPPH solution) and the DPPH containing plant extract solution.

2.6.2. Ferric reducing power assay
Ferric reducing antioxidant power (FRAP) of plant extracts was determined as per the procedure described by Do et al. (2014). Briefly, from the stock solution (1 mg/ml), five serial concentrations of extracts (200, 150, 100, 50, 25 mg/ml) were prepared; mixed with phosphate buffer (2 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 10%). After the solutions were incubated at 40°C for 30 min, trichloroacetic acid (2.5 ml, 10%) was added and the tubes were centrifuged at 3000 rpm for 10 min. Then, a supernatant (5 ml) of each solution was mixed with distilled water (2 ml) and ferric chloride (0.5 ml, 0.1%). Finally, the absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control. Ferric reducing power was expressed as absorbance per specific amount of plant extract.

2.6.3. Statistical analysis
The obtained data were statistically analyzed using multivariate analysis of SPSS (version 20) (SPSS Inc., Chicago, USA) at 95% confidence level. All the experiments were done in duplicate and the results were expressed as Mean± SD. Mean comparisons were considered statistically significant when \( p \leq 0.05 \).

3. Results and discussion

3.1. Percentage extraction yield
As the chemistry of various bioactive compounds in medicinal plants varied, different solvents of extraction may have different effects on the solubility, extraction yield and antioxidant activity of the phyto-compounds (Azabou et al., 2020). In the present study, the percentage
extraction yield of seven different crude solvent extracts (ethanol, methanol, 80% ethanol, 80% methanol, ethyl acetate, dichloromethane and petroleum ether) prepared from C. rotundifolia leaf was calculated and presented in Table 1. From the statistical data obtained, the means value of percentage extract yield showed a significant difference (P ≤ 0.05) between each solvent of extractions. Among the solvent of extractions used in this study, 80% aqueous ethanol scored the highest extraction yield (16.22%) followed by 80% aqueous methanol (12.90%), methanol (12.87%) and ethanol (12.86%); whereas the lowest crude extract percentage yield was recorded by petroleum ether (1.11%) followed by ethyl acetate (1.42%) and dichloromethane (1.65%). The obtained results indicated that polar protic solvents gave better extraction yields and this may tell us that the plant is enriched with polar secondary metabolites which was slight bit in agreement with previously reported results on the same plant species (Al-Fatimi et al., 2007). According to this author, the maximum extract yield was obtained from MeOH (14.5%) and water (11.2%) extracts; while the lowest yield was recorded by dichloromethane extract (3.2%). In the same way, aqueous methanol is one of the most promising potential in recovering the highest amounts of phenolic compounds (Sultana et al., 2009). This indicated that the extraction yield is not only affected by the extraction technique but also by the extraction solvent. Furthermore, using hydroalcoholic as solvent of extraction may facilitate the exploitation of chemical components as they may be soluble either in water and/or organic solvent parts (Do et al., 2014; Truong et al., 2019). In this study also, this rational may be the reason why the aqueous ethanol and methanol solvents scored the highest extract yields than the pure ethanol and methanol, and the rest solvent of extractions. Therefore, ethanol, methanol and their corresponding aqueous solvents could be preferred for further investigation on the biological activity and isolation of effective bioactive compounds of C. rotundifolia leaf.

3.2. Preliminary phytochemical profiling of crude extracts
In this study, the chemical profiles of all crude extracts prepared from C. rotundifolia leaf were screened using different reagents to confirm the absence/or presence of major phytochemical classes. Accordingly, eight phyto-compound families, namely, flavonoids, phenols, alkaloids, tannins, saponins, steroids, terpenoids and carbohydrates were screened as depicted in Table 2.

As it can be seen from Table 2, the obtained preliminary qualitative analyses results revealed that flavonoids, phenols and saponins were strongly detected in EtOH, MeOH and their aqueous solvent crude leaf extracts of C. rotundifolia. Whereas tannin was moderately screened in all extracts, except in petroleum ether was negative. According to the previous study reported by Al Hamoud et al. (2019), ethanolic extract of aerial parts of same plant species showed the presence of flavonoids which is in line with the present result. Also in this study, alkaloids and tannins were observed strongly and moderately, respectively, in dichloromethane extract. All extracts, except ethanol and methanol, showed a positive presence of carbohydrates (Table 2). On the contrary, steroids and terpenoids were completely absent in all tested crude plant extracts. From the present result, it is possible to say that the ethno-medicinal value of

| Table 1. Percentage yields of seven different crude extracts of Cadoba rotundifolia leaf |
|----------------------------------|-------------------------|---------------------|
| Solvent system                  | Extraction yield (%)    |
| Petroleum ether                 | 1.11 ± 0.06             |
| Dichloromethane                 | 1.65 ± 0.06             |
| Ethyl acetate                   | 1.42 ± 0.09             |
| Ethanol (99.5%)                 | 12.86 ± 0.30            |
| Methanol (99.8)                 | 12.87 ± 0.20            |
| Aqueous ethanol (80%)           | 16.22 ± 0.30            |
| Aqueous methanol (80%)          | 12.90 ± 0.21            |

Results are mean ± SD, n = 3.
C. rotundifolia leaf such as treating cough, stomach ulcer and cancer (Al-Fatimi, 2019) might be attributed to the positively screened secondary metabolites such as alkaloids, flavonoids and tannins (Table 2).

3.3. Estimation of total phenolics, flavonoids and tannins contents

The total phenolic content (TPC) in the ethanolic, methanolic and their aqueous (80%) crude extracts of C. rotundifolia leaf were presented in Table 3. The obtained results ranged from 10.46 to 8.04 mgGAE/gDCE which was determined from the linear regression equation of standard curve (y = 0.007x−0.009, R² = 0.996). From the statistical data obtained, the difference in mean value between each extract was generally insignificance (p ≥ 0.05) except between extracts 1 and 4 (P ≤ 0.5). As shown from Table 3, the highest content of total phenolics (mgGAE/gDCE) was detected in 80% methanol extract (10.46) followed by 80% ethanol (9.63) and pure methanol (9.54), while the lowest total phenolic content was recorded in pure ethanol (8.04). The results generally confirmed that the hydroalcoholic crude extracts provide satisfactory phenolic content. According to Anwar and Przybylski (2012), aqueous methanol and ethanol solvent extracts were found to be more effective for isolation and determination of phenolic compounds from different plant materials; and this statement is a confirmation for our findings reported in the present study.

The total flavonoid content (TFC) (mgCE/gDCE) in the tested solvent extracts was obtained using the equation of standard curve (y = 0.028x +0.008; R² = 0.998). The highest and lowest TFC values were ranged from 0.51 ± 0.04–0.38 ± 0.05 mgCE/gDCE (Table 3). The resulted statistical output indicated that the mean value between tested solvent extracts showed a significantly different (p ≤ 0.05), except between extracts 1 & 2 and 3 & 4 which was insignificance (p ≥ 0.05).

### Table 2. Phytochemicals profile from different crude extracts of Cadaba rotundifolia leaf

| Phytochemical components | Pet. ether | DCM | EtOAc | EtOH | MeOH | 80% EtOH | 80% MeOH |
|--------------------------|------------|-----|-------|------|------|----------|----------|
| Flavonoids               | -          | -   | -     | ++   | +    | ++       | +        |
| Phenols                  | -          | ++  | +     | +    | -    | ++       | -        |
| Alkaloids                | -          | ++  | +     | +    | -    | ++       | -        |
| Tannin                   | -          | +   | +     | +    | +    | +        | -        |
| Saponins                 | -          | -   | -     | ++   | +    | ++       | +        |
| Steroids                 | -          | -   | -     | -    | -    | -        | -        |
| Terpenoids               | +          | +   | +     | +    | -    | +        | +        |
| Carbohydrates            | +          | +   | +     | +    | -    | +        | +        |

Note: ++ = strong presence, + = moderate presence,— = absence

### Table 3. Total phenolic, flavonoid and tannin contents in four different crude leaf extracts of Cadaba rotundifolia

| S. no. | Crude extracts       | Total phenolics (mgGAE/gDCE) | Total flavonoids (mgCE/gDCE) | Total tannin (mgCE/gDCE) |
|--------|----------------------|------------------------------|-----------------------------|--------------------------|
|        | Ethanol (99.5%)      | 8.04 ± 1.04                  | 0.38 ± 0.05                 | 0.81 ± 0.01              |
|        | Methanol (99.8)      | 9.54 ± 0.1                   | 0.41 ± 0.02                 | 0.75 ± 0.03              |
|        | Aqueous ethanol (80%)| 9.63 ± 1.02                  | 0.46 ± 0.02                 | 1.05 ± 0.03              |
|        | Aqueous methanol (80%)| 10.46 ± 1.25              | 0.51 ± 0.03                 | 0.92 ± 0.03              |

Results are mean ± SD, n = 3.
obtained results showed that the aqueous methanol and ethanol extracts exhibited the highest TFC value (0.49 ± 0.04 and 0.46 ± 0.02 mgCE/gDCE, respectively). As shown in Table 3, the total content of tannin was calculated in the crude extracts from the standard calibration curve of catechin concentrations ($y = 0.005x+0.011$, $R^2 = 0.997$). According to the obtained results, the highest total tannin content was recorded in 80% ethanol extract (1.05 mgCE/gDCE) followed by 80% methanol (0.92 mgCE/gDCE). Nevertheless, the obtained total tannin content in general did not show a significant difference between the tested crude extracts ($p ≥ 0.05$).

3.4. In-vitro anti-DPPH radical activity of Cadaba rotundifolia crude leaf extracts
In the present study, the anti-free radical scavenging potential of four crude extracts of C. rotundifolia leaf against DPPH free radical was evaluated. Ascorbic acid and plant extract free DPPH solution were used as positive and negative controls, respectively. The obtained DPPH free radical scavenging percentage of the tested crude extracts together with the ascorbic acid is presented in Figure 2.

The obtained output of statistical analysis showed that the difference in anti-DPPH activity mean values between each tested extracts was significance ($p ≤ 0.05$) except between extracts 1 & 4 ($p ≥ 0.05$). As it can clearly be seen from Figure 2, 80% methanol extract exhibited the greatest DPPH radical scavenging activity followed by 80% ethanol. The lowest scavenging percentage was recorded by the non-aqueous ethanol extract followed by methanol analogues. Overall, the methanol family was found to be the better extract than the ethanol family in the trapping of DPPH free radicals. According to many scholars, for example Škerget et al., 2005, the anti-DPPH free radical trapping potential of plant extracts is mainly due to the presence of high contents of polyphenolic and flavonoid compounds which easily donate free hydrogen radical. This fact definitely supports our findings, in which the aqueous part of the methanol and ethanol crude extracts which scored the highest total phenolic, and flavonoid contents also exhibited the highest DPPH free radical scavenging percentage than the non-aqueous parts.

3.5. Ferric reducing antioxidant power (FRAP)
The reducing potential of chemical entities found in a given analyte such as plant extract could be evaluated by monitoring the reduction of ferric ($Fe^{3+}$) into the ferrous ($Fe^{2+}$) form. That is,
ferric ions \((Fe^{3+})\) originated from ferricyanide complex \((K_3Fe(CN)_6)\) can be reduced to the ferrous ions \((Fe^{2+})\) by observing the change in colour of the reaction solutions from yellow to green in the presence of antioxidants. The produced \(Fe^{2+}\) concentration is monitored by measuring its absorbance at 700 nm (Anwar & Przybylski, 2012; Sun et al., 2015). As shown from Figure 3, the ferric reducing power of the tested plant extracts was positively correlated with their concentrations. The higher colour intensity of the mixtures revealed greater absorbance and higher reducing activity or antioxidants (the presence of more reductants) in the crude extracts. The hydroalcoholic (80% MeOH and 80% EtOH) crude extracts of \(C.\ rotundifolia\) leaf showed greater FRAP than the corresponding non-aqueous ones (Figure 3). This result, like that of DPPH assay, showed a positive correlation between the ferric reducing antioxidant power (FRAP) and the total content of phenolics and flavonoids (Table 3) the hydroalcoholic crude extracts recorded. The FRAP mean value between each evaluated extract was significantly different \((p \leq 0.05)\) according to the statistical data obtained.

4. Conclusion

As the chemistry of phyto-compounds varies, different solvents of extraction may have different effects on the solubility, extraction yield and antioxidant activity of the phyto-compounds. In the present study, among the solvent of extraction used, 80% aqueous ethanol scored the highest extraction yield followed by 80% aqueous methanol, methanol and ethanol. Therefore, ethanol, methanol and their corresponding aqueous solvents could be preferred for further investigation on the biological activity and isolation of effective bioactive compounds of \(C.\ rotundifolia\) leaf. Regarding the phytochemical screening test, major phytochemical classes such as flavonoids, phenolics, alkaloids, tannins, carbohydrates and saponins were positively observed in different crude extracts of \(C.\ rotundifolia\) leaf. It is, therefore, possible to say that the reported ethno-medicinal value of \(C.\ rotundifolia\) leaf might be attributed to such positively screened secondary metabolites. Quantitatively, the hydroalcoholic crude extracts (80% methanol and 80% ethanol) of \(C.\ rotundifolia\) leaf showed better total phenolics, flavonoids and tannins content; and such results were also correlated with the DPPH anti-free radical scavenging activity and ferric reducing antioxidant power (FRAP) of the mentioned extracts. In general, the overall findings obtained from the present study pave a way for the upcoming extensive research works which might be conducted on the pharmacological assay and phyto-compound investigations of \(C.\ rotundifolia\) leaf. From the present findings, it is also very important to note that the hydroalcoholic solvents should be prioritized while conducting any phyto-pharmacological studies on the \(C.\ rotundifolia\) leaf, in the near future.
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