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Mineral composition, phenolic profile, antioxidant and antimicrobial activities of *Corchorus depressus* roots extracts

T. H. Bokhari[1*], M. A. Aslam[1], S. Hina[2], N. B. Rizvi[3], N. Rasool[1], M. J. Saif[4], M. Zubair[1], A. I. Hussain[4], S. A. Shahid chatha[4], M. Raiz[1]

[1]*Department of Chemistry, Government College University, Faisalabad, Pakistan*
[2]*Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan*
[3]*Institute of Chemistry, New Campus, University of the Punjab, Lahore*
[4]*Department of Applied Chemistry and Biochemistry, Government College University, Faisalabad, Pakistan*

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The present study was carried out to examine the mineral contents, phenolic profile, antioxidant and antimicrobial activities of *Corchorus depressus* roots extracts. The ground roots were extracted with the following solvents: n-hexane, ether, acetone, ethanol and methanol. The *Corchorus depressus* roots extracts contained the following metals: cobalt, nickel, copper, chromium, zinc, lead, and iron. Total phenolic contents (9.64-40.14 mg/100 g of dry matter were measured as Gallic Acid Equivalent), and total flavonoid contents (8.08-37.04 mg/100 g of dry matter) were measured as Catechin Equivalent). *Corchorus depressus* roots extracts showed very good DPPH radical scavenging of 56.31 % inhibition. The GC-MS analysis of the essential oils from the roots showed 13 compounds with n-butyl acetate (12.48%), 2,3,3-trimethyl octane (16.36%) and *cis*-methyl hexadecimal compounds (9.15%) as major components. All results of *Corchorus depressus* roots extracts and essential oils demonstrated significant ($P < 0.05$) variations.

Keywords: *Corchorus depressus*, DPPH, total phenolics, total flavonoids, MIC, GC-MS.

INTRODUCTION

An important phenomenon attracting the attention of many scientists is oxidation. Free radicals are considered to initiate oxidation which leads to aging and causes diseases in human beings. Oxidation widely occurs in food systems. It is mediated by oxygen free radicals or reactive oxygen species. Among organic compounds, lipids are prone to auto-oxidation reactions with oxygen. Lipid peroxidation is a major reaction of food deterioration which is responsible for significant changes in texture and nutritive value. Excessive amounts of free radicals are produced during the oxidation process with contribute to the progression of many clinical diseases. Dietary antioxidants might play positive role in delaying or inhibiting oxidation reactions [1].

Antioxidants play an important role in scavenging free radicals both *in vivo* and *in vitro*. The use of synthetic antioxidants such as butylated hydroxyltoluene, butylated hydroxylanisol, propylgallate and *tert*. butylhydroquinone promotes negative health effects [2].

There is worldwide interest in replacing synthetic antioxidants with natural ones. It has been observed that synthetic antioxidants are carcinogenic, pathogenic and toxic. Enzymes, lipids and reproductive processes are affected by them [3].

At present, plant-based natural antioxidants like phenols, flavonoids and tocopherols are gaining high recognition due to their antioxidant activities. They show anti-carcinogenic potential and various health-promoting effects. Food manufacturers prefer natural antioxidants to synthetic antioxidants as additives to healthy foods [4].

Some earlier chemical analyses and biological studies of various plants have been made [5-9] and more research work is required to study the unexplored plants.

Corchorus depressus belongs to the family *Mavaceae* (formally under *Tiliaceae)* and the genus is *Corchorus*. The common name of this plant is Bauphali. It is a perennial herb. It occurs in Pakistan, India, Africa and Cape Verde Islands. It grows from sea level to an altitude of 1000 m in arid and semi-arid regions throughout Pakistan [10]. It is 6-9 inches in length. The roots are diffusely branched. Its leaves are ecliptic, 4-18 mm long and 2-9 mm wide. Its flowers are yellowish, 1 mm long. The growth of leaves and fruits is stunted in saline and rocky soils [11].This plant is regarded

* To whom all correspondence should be sent:
E-mail: tanveer.bokhari@yahoo.com

as a good sand binder in the desert. Its seeds are minute and their color is like chocolate. Its fruits are capsule-like with length in the range of 8-15 mm. Its branches are radiatingfrom a woody crown [12]. It is reported that *Corchorus depressus* contains triterpenoids, sterols, phenolics, fatty acids, cardiac glycosides and carbohydrates [13]. Approximately 100 species of *corchorus* are found. South Africa is richest in species of *corchorus* (16), followed by Tanzania (13 species), Ethiopia (12 species), Kenya (11 species) and Pakistan (6 species). Wild species are mostly found in Africa, America, Brazil, Mexico, Bolivia, Venezuela, West Indies, Australia, China, Taiwan, India, Japan and Sri Lanka [14].

EXPERIMENTAL

Collection of Sample

Corchorus depressus roots were collected from Nankana District, Pakistan. The sample was identified and authenticated by Dr. Muhammad Naeem, Assistant professor in the Botany Department of Government College University Faisalabad, Pakistan. The collected roots were dried under ambient temperature and crushed to powder in a mortar.

Chemicals and Reagents

Gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (90.0 %), folin-ciocalteu reagent, butylated hydroxytoluene (99.0 %), linoleic acid, ascorbic acid, aluminum chloride, ferric chloride, ferrous chloride, sodium nitrite, trichloroacetic acid, potassium ferricyanate were purchased from Sigma Chemicals Co (St, Louis, MO, USA). All other analytical grade chemicals such as ammonium thiocyanate, methanol and anhydrous sodium carbonate were obtained from Merck (Darmstadt, Germany).

Preparation of Corchorus depressus Extract

Extracts of *Corchorus depressus* roots were prepared using solvents of increasing polarity: methanol, ethanol, acetone, ether and n-hexane.

Determination of Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Folin-ciocalteu reagent was used to determine the total phenolic contents following an already reported method [15].

Antioxidant activity in a linoleic acid system

The antioxidant activity was determined in terms of percentage inhibition of the peroxidation of a linoleic acid system using a method reported by Riaz *et al.* and Yen *et al.* [16,17]. The percentage inhibition of linoleic acid peroxidation was calculated by the following equation:

% inhibition of peroxidation = 100-[(Abs. increase of sample at 175h/Abs. increase of control at 175h) × 100].

DPPH Free Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was carried out spectrophotometrically as reported in [18]. The IC_{50} values were calculated. Three replicates were recorded for each sample. The percentage scavenging activity was calculated by the following equation:

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

Reducing Power

The reducing power of the extracts was determined by the method reported in [16,17]. The plant extracts containing 2.5-10 mg/ml of dry matter were mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0 %); the mixture was incubated at 50°C for 20 min. Then 5 mL of 10 % trichloroacetic acid was added, centrifuged at 980x*g* for 10 min at 5°C in a refrigerated centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water. 1 mL of 0.1 % ferric chloride was added and the absorbance was measured at 700 nm.

Antimicrobial Activity

The antimicrobial activity of the plant extracts was studied against the bacterial strains, *Pasturella multocida* (locally isolated), *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and the fungal strains *Aspergillus niger*, *Aspergillus flavus*, *F. solani* and *Rhizopus solani* by the disc diffusion method [19]. The minimum inhibitory concentration (MIC) was determined as described in [20,21] with some modifications.

Statistical Analysis

The experiments were carried out in triplicate and statistical analysis of the data was performed by analysis of variance, using STATISTICA 5.5. The probability value $p \leq 0.05$ was considered to denote a statistically significance evaluation. All results were presented as mean ± SD.

RESULTS AND DISCUSSION

Percentage yield of roots extracts

The present study shows that the percentage yield extracts in the different solvents was in the range of 2.89 -5.69% in (Table 1)

Table 1. Percentage yield of different extracts of *corchorus depressus* roots

Extracts of roots	% Yield (g/100g dry matter)
Methanol	5.69
Ethanol	5.27
Acetone	3.29
Ether	3.19
n-Hexane	2.89

Total Phenolic Contents (TPC)

The folin-ciocalteu method was used for the determination of the quantity of TPC in the *Corchorus depressus* extracts. This method was preferred because of its higher sensitivity, lower interference and rapidity in comparison with other test [22]. Table 2 presents the amounts of TPC (mg/100 mg of dry weight as GAE).

Table 2. Total phenolic contents (TPC) of *Corchorus depressus* roots

Extract	TPC (mg/100 g of dry matter measured as GAE)
Methanol	40.14±1.02
Ethanol	40.32±1.07
Acetone	26.62±0.06
Ether	11.20±1.02
n-Hexane	9.64±0.07

The present results for TPC in *Corchorus depressus* roots using pure methanol as a solvent were in close agreement with those (38.60 mg GAE/100g fresh weight) reported for the antioxidant activity of phenolic fractions in *Corchorus depressus* roots in methanol using an FTC model (HUDA) [23].

Table 3 presents the amount of total flavonoid contents (mg/100 g of dry weight as CE) of the roots extracts of *Corchorus depressus*. The present results for TFC of *Corchorus depressus* roots are in the range of 8.08-37.04 (mg/100g of CE).

Table 3. Total Flavonoid Contents (TFC) of *Corchorus depressus* roots

Extract	TFC (mg/100 g dry matter measured as (CE)
Methanol	37.04±0.07
Ethanol	28.14±1.02
Acetone	26.07±0.09
Ether	24.34±1.03
n-Hexane	8.08±0.06

DPPH Radical Scavenging Activity

The free radical DPPH is stable and has a deep violet color in the range of 515-528 nm. As DPPH accepts a proton from proton-donating species, especially phenols, its color changes from deep violet to yellow. The DPPH scavenging activity increases as the concentration and degree of hydroxylation increases. In the transformation of DPPH to its reduced form DPPH-H, the extracts behave as donors of protons or electrons. In a concentration- dependent manner, the free radical scavenging capacity of the extracts increased. Table 4 shows that the *Corchorus depressus* root extracts display excellent radical scavenging activity.

Table 4: DPPH free radical scavenging activity of *Corchorus depressus* roots

Extract	$IC_{50(\mu g/ml)}$
Methanol	69.01±0.09
Ethanol	68.04±0.12
Acetone	58.06±0.16
Ether	36.11±0.12
n-Hexane	19.23±0.16
BHT	76.30±0.08

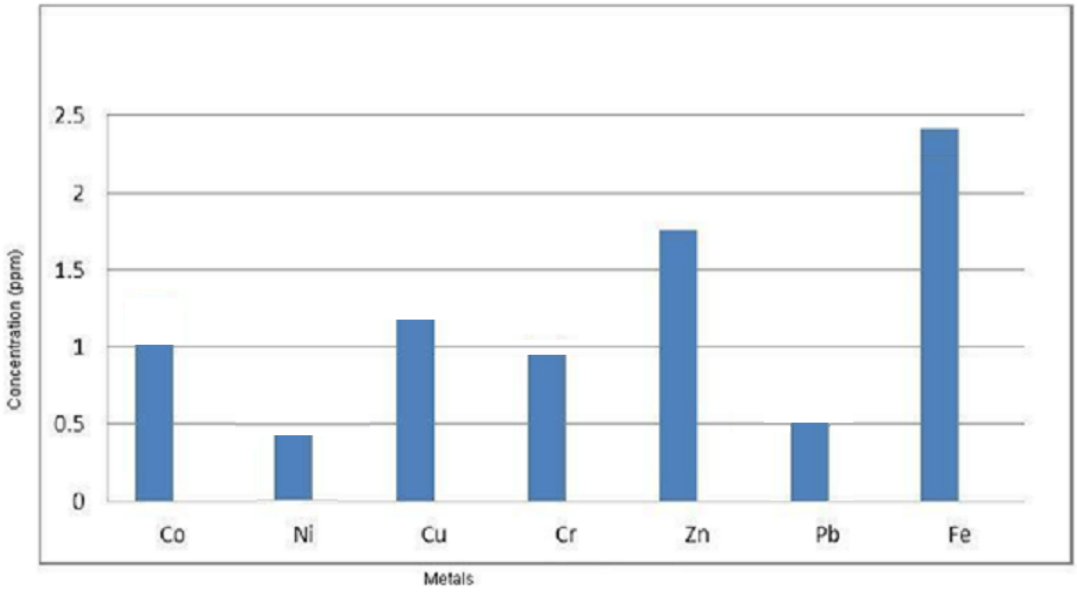

Fig. 1 Concentration of metals in *Corchorus depressus* roots

Pure methanol exhibits the highest scavenging activity but slightly lower antioxidant activity when compared with the synthetic antioxidant BHT. Our result is comparable with those reported for the plant extract [18]. The scavenging activity of *Corchorus depressus*, was significantly ($p<0.05$) lower than that of BHT which showed an IC_{50} value of 19.23 µg/mL.

Percentage Inhibition of Peroxidation in Linoleic Acid System

As antioxidants have the ability to prevent oxidation, the percentage inhibition of linoleic acid was employed to check the antioxidant activity of *Corchorus depressus* roots extracts, (Table 5). Linoleic acid gives upon oxidation peroxides which oxidize Fe^{+2} to Fe^{+3}. According to statistical analysis, the variation in percentage inhibition of the oxidation in linoleic acid by roots extracts was significant ($p<0.05$) depending on the solvent system. The % inhibition of *corchorus depressus* using absolute methanol solvent system was found to be comparable with that of the synthetic antioxidant BHT (93.09%). Our results are in good agreement with the reported value of up to 70.60% for linoleic acid inhibition of peroxidation [24].

Concentration of metals in *Corchorus depressus* roots are presented in Fig. 1.

Table 5: Percentage inhibition of linoleic acid peroxidation by *Corchorus depressus* roots

Extract	% Inhibition
Methanol	56.31±1.03
Ethanol	49.71± 1.06
Acetone	42.32±0.09
Ether	16.20±0.06
n-Hexane	11.24±1.02
BHT	93.09±1.6

Reducing Power

The trends in the reducing power of *Corchorus depressus* roots extracts are presented in Fig. 2; the greater the color intensity, the greater is the absorption and, correspondingly, the antioxidant activity. The absorbance of the sample was measured out spectrophotometrically; the antioxidant activity increased when the concentration of the extracts increased. The extracts having higher concentration of phenolics and higher antioxidant activity also showed good reducing power of the phytochemical constituents [25]. Therefore, the reducing power may be used in the evaluation of antioxidant activity of plant extracts.

Antimicrobial Activity

The antimicrobial activity of the extracts against selected microorganisms is presented in Tables 6-7. The *Corchorus depressus* roots showed significant antimicrobial potential against most of the fungal and bacterial strains ($p< 0.05$). The *n*-hexane fraction showed the lowest activity against all tested fungal strains with zones of inhibition (mm) against *B. subtilis* (7.21 mm), *S. aureus* (6.18 mm), *P. multocida* (9.73 mm), *E. coli* (9.71 mm) respectively. The results indicated that the methanol extracts showed good activity against fungal strains. The lowest inhibition zones were registered against *F. solani* (15.44 mm). The *n*-hexane fraction showed the lowest activity against all tested fungal strains with zones of inhibition against *R. solani* (9.08 mm), *F. solani* (7.44 mm), *A. flavus*(8.66 mm) and *A. niger* (6.92 mm). It was

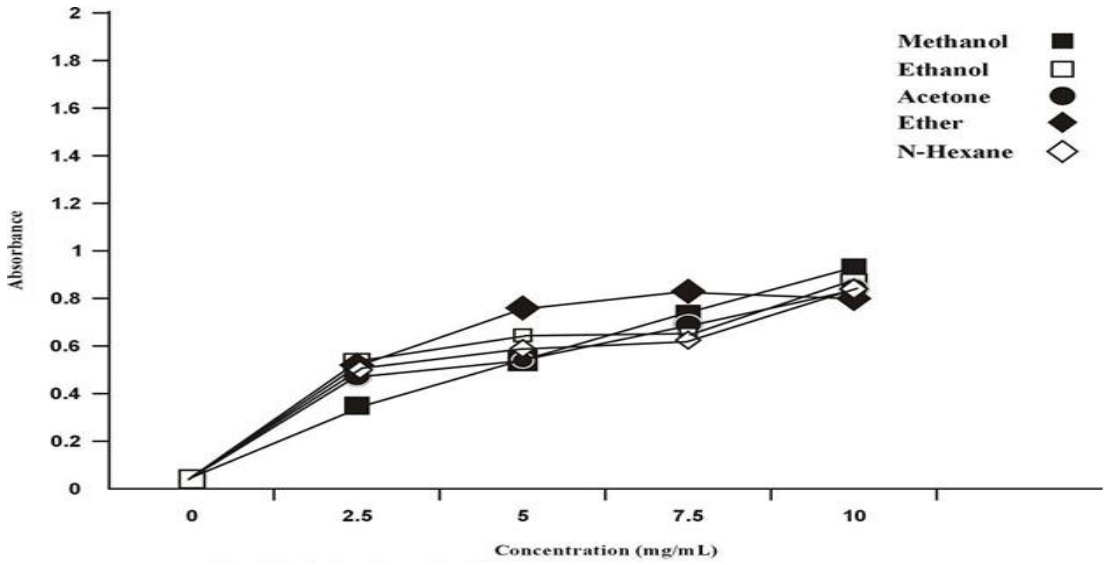

Fig: 2 Reducing potential of *Corchorus depressus* roots extracts

earlier reported that the phytochemical constituents in plants, which are active against microorganisms, are aromatic or saturated organic compounds, and are most often obtained through methanol extraction [26,27]. Refampicine and terbinafine were used as positive controls for bacterial and fungal strains, respectively. The standard showed higher activity on the organisms than the tested extracts (Tables 6-7). In our studies all extracts (methanol, ethanol, acetone, ether and n-hexane) were active against the tested fungal strains and methanol extract of *Corchorus depressus* roots was used to cure diseases caused by bacterial and fungal strains in the present research. The plants may contain organic compounds like steroids, tannins,

Table 6: Antifungal activity of *Corchorus depressus* roots by zone of inhibition determination (mm)

Extracts	*A. niger*	*A. lavus*	*F. solani*	*R. solani*
Methanol	18.24±0.16	15.62±0.21	15.44±0.41	14.62±o.15
Ethanol	16.29±0.29	14.86±0.26	12.26±0.37	13.86±0.26
Acetone	13.54±0.17	13.22±0.31	11.06±0.26	12.62±0.23
Ether	13.24±0.24	14.24±0.24	9.12±0.28	11.12±0.16
n-Hexane	6.92±0.51	8.66±0.29	7.44±0. 13	9.08±0.39
Terbinafine	18.56±0.44	19.16±0.5	20.16±0.45	18.24±0.31

Table 7: Antibacterial activity of *Corchorus depressus* roots by zone of inhibition determination (mm)

Extracts	*P. multocida*	*E. coli*	*B. subtilus*	*S. aureus*
Methanol	17.41±0.11	16.21±0.16	15.06±0.02	14.22±0.07
Ethanol	14.23±0.24	15.46±0.19	13.47±0.03	13.66±0.02
Acetone	14.48±0.62	13.24±0.26	11.66±0.17	11.28±0.03
Ether	12.66±0.33	11.66±0.24	8.43±0.12	10.24±0.14
n-Hexane	9.73±0.06	9.71±0.07	7.21±0.04	6.18±0.04
Rifampicine	20.82±0.13	21.14±0.52	21.63±0.48	19.82±0.51

Table 8: Antibacterial activity of *Corchorus depressus* roots by minimum inhibitory concentration (MIC, mg/mL)

Extracts	*P. multocida*	*E. coli*	*B. subtilus*	*S. aureus*
Methanol	0.065±0.03	0.084±0.02	0.052±0.02	0.062±0.002
Ethanol	0.072±0.02	0.076±0.03	0.061±0.02	0.058±0.002
Acetone	0.044±0.02	0.065±0.12	0.071±0.04	0.047±0.004
Ether	0.041±0.04	0.019±0.16	0.047±0.12	0.049±0.03
n-hexane	0.043±0.05	0.016±0.13	0.018±0.14	0.039±0.002
Rifampicine	0.62±0.02	0.92±0.02	0.52±0.003	0.065±0.003

Table 9: Antifungal activity of *Corchorus depressus* roots by minimum inhibitory concentration (MIC, mg/mL)

Extracts	*A. niger*	*A. flavus*	*F. solani*	*R. solani*
Methanol	0.176±0.006	0.058±0.005	0.056±0.002	0.076±0.004
Ethanol	0.162±0.005	0.082±0.005	0.047±0.004	0.062±0.002
Acetone	0.153±0.004	0.147±0.004	0.052±0.003	0.059±0.003
Ether	0.154±0.005	0.141±0.004	0.039±0.004	0.047±0.004
n-Hexane	0.121±0.007	0.151±0.002	0.092±0.002	0.037±0.006

Table 10: Gas chromatographic and mass spectral data for the essential oil from *Corchorus depressus* roots

Sr. No	Retention time(min)	Compound Name	% Composition
1	6	P-xylene	2.89
2	7	n-Butylacetate	12.48
3	7.65	2,4,6 Trimethylheptane	5.06
4	9.40	2,3,3, Trimethyloctane	16.36
5	11.55	5- Methyloctadecane	2.61
6	12.35	4- Chloro-2,6-dioxaadmantane	2.33
7	13.80	Plamitic acid, methyl ester	3.36
8	14.94	Cis-9- hexadecenal	9.15
9	17.16	Roridin A	1.12
10	17.35	Tetradecamethylhexasiloxane	0.56
11	20.13	Lupeol	1.44
12	22	Octadecamethyl	0.84
13	24	Uridine	0.71

saponins, flavonoids, alkaloids, anthraquinone. These compounds have been reported to exhibit antimicrobial activity in [3]. The phytochemicals may have antimicrobial property through different mechanisms. The antimicrobial studies performed in [28] showed that the extracts tested were active against the fungal strains. These observations support the use of *Corchorus depressus* in herbal cure remedies. The traditional medicine uses this plant in the treatment of various diseases [29].Table 8 and 9 represent antibacterial and antifungal activities of Corchorus depressus roots by minimum inhibitory concentration (MIC, mg/mL) respectively.

GC-MS Analysis of Corchorus depresuss roots essential oil

GC-MS analysis of the essential oil from the stem was performed. The chemical composition of *R. equisetiformis* essential oils is presented in Table 10. The major compounds with their percentage are 2,3,3-trimethyloctane (16.36 %), n-butyl acetate (12.48 %), cis-9-hexadecenal (9.15%). It was found that the biological activities such as antioxidant potential sre similar to those of some phyto-components [10,29].

CONCLUSION

The *Corchorus depressus* extracts and essential oil showed significant antioxidant potential in terms of scavenging free radicals. The extracts showed antimicrobial properties against selected bacterial and fungal strains. The roots contain some metals with maximum content of iron.

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МИНЕРАЛЕН СЪСТАВ, ФЕНОЛЕН ПРОФИЛ, АНТИОКСИДАНТНА И АНТИМИКРОБНА АКТИВНОСТ НА ЕКСТРАКТИ ОТ КОРЕНИ НА *Corchorus depressus*

Т.Х. Бохари[1*], М.А. Аслам[1], С. Хина[2], Н.Б. Ризви[3], Х. Расуул[1], М. Дж. Саиф[4], М. Зубаир[1], А.И. Хусаин[4], Ш.А.Шахид Чатха[4], М. Риаз[1]

[1]*Департамент по химия, Правителствен университетски колеж, Файсалабад, Пакистан*
[2]*Департамент по биоинформатика и биотехнология, Правителствен университетски колеж, Файсалабад, Пакистан*
[3]*Институт по химия, Нов кампус, Университет в Пунджаб, Лахор, Пакистан*
[4]*Департамент по приложна химия и биохимия, Правителствен университетски колеж, Файсалабад, Пакистан*

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(Резюме)

В настоящата работа се изследват минералният състав, фенолен профил, антиоксидантна и антимикробна активност на екстракти от корени на *Corchorus depressus.* Корените се третират със следните екстрагенти: *n*-хексан, етер, ацетон, етанол и метанол. Екстрактите от корените на *Corchorus depressus* съдържат следните метали: кобалт, никел, мед, хром, цинк, олово и желязо. Общото фенолно съдържание е между 9.64-40.14 mg/100 g сухи вещества като еквивалент на галова киселина, а общото съдържание на флавоноиди (8.08-37.04 mg/100 g сухи вещества) е измерено като катехинов еквивалент. Екстрактите от корените на *Corchorus depressus* показват много добра DPPH радикал-премахваща активност, като 56.31 % инхибиране. GC-MS-анализът на есенциалните масла от корените притежават 13 съединения с n-бутилацетат (12.48%), 2,3,3-триметил октан (16.36%) и *cis*-метил хексадецимални съединения (9.15%) като главни компоненти. Всички резултати за екстрактите от корени на *Corchorus depressus* и есенциални масла показват значителна дисперсия ($P < 0.05$).