

Original Research Article

Free-Radical Scavenging Assay and Quantitative Estimation of Flavonoids from *Corchorus depressus*

Suggestion for Title: Antioxidant activity and total flavonoids quantitative analyses of polar extracts of *Corchorus depressus* leaves

ABSTRACT

Background: The investigation of ~~chemical constituents (Qualitative & Quantitative)~~ total flavonoids and ~~the evaluation of the~~ antioxidant activity of polar extracts of *Corchorus depressus* is the major aim of this study. As observed from ancient literatures and folkloric claims the plant *Corchorus depressus* worshipped by the married women of Odisha, India, in the rituals called as “Jama Jutia”, possesses different biological activities including antioxidant property.

Methods: The DPPH (diphenyl picryl hydrazine), hydroxyl radical (OH) and nitric oxide (NO) radical scavenging methods were performed for measurement of the antioxidant activity at different extracts. The flavonoid rich fraction and phenolic content of the extracts were determined by using aluminium chloride and Folin-Ciocalteu’s reagent (FCR) methods respectively.

Results: The results for estimation of total phenolic content (mg/100g) expressed as gallic acid equivalent (GAE) and total flavonoid (mg/100g) in weight of quercetin equivalent (QE) was highest in methanolic extract 78.46 and 21.2 respectively, followed by 18.18 mg/100g in GAE and 1.80mg/100g in QE for aqueous extract.

1 **Conclusion:** The methanolic extract of *C. depressus* (MECD) at 100µg/ml showed highest
2 DPPH, OH and NO radical scavenging activity and this activity may be attributed due to the
3 presence of saponins and flavonoids as detected in the extract.

4
5 Key words: Antioxidant, Flavonoids, *Corchorus depressus*, Hydroxyl radical

6 7 8 1. INTRODUCTION

9 A majority of the present-day diseases are reported to be due to the shift in the balance of the
10 pro-oxidant and the anti-oxidant homeostatic phenomenon in the body. Pro-oxidant conditions
11 dominate either due to the increased generation of free radicals caused by excessive oxidative
12 stress of the present day life or due to the poor scavenging or quenching effect in the body
13 caused by depletion of the dietary anti oxidant low ingestion of anti-oxidant compounds [1-2].

14 A lot of synthetic antioxidants like BHT butylated hydroxytoluene, BHA butylated
15 hydroxyanisole and gallates are reported to produce cancer are reported as potential
16 carcinogens and hence their use have been restricted, as such there is an increased demand for
17 the search of natural products that can be utilized as an antioxidant [3].

18 Plants are considered as wide source of antioxidants. The polyphenolic compounds like
19 flavonoids present in them are excellent antioxidants. The proton donating ability property of
20 polyphenols empowers it to interrupt the oxidation mechanism so there is prevention of
21 oxidative damage [4-5].

22 The strong biological activity of Plant phenolics and flavonoids outlines their necessity for
23 quantitative determination.

1 *Corchorus depressus* (Linn.) C. Christensen [family *Tiliaceae*]. ←

2 <https://plants.jstor.org/stable/10.5555/al.ap.flora.fwta1673>

3 The selected plant *Corchorus depressus* Linn. (Tiliaceae) is treated as religious and
4 worshipped by the married women of Odisha, India, in the rituals called as “Jama Jutia”. The
5 women make a daylong fasting, prepare different sweets, cakes and worship the plant in the
6 evening. An old man of the society will sit at a distance and act as “Yamaraj” (The Lord of
7 Death). The women sweep the road with the plants up to the old man and offer the cakes. The
8 worshipped plants were taken up by the women and softly swept over on the body of their
9 family members and it is believed that by doing so the family members will be free from attack
10 of any disease and have a long life (**Figure 1**).

11



12

13 **Figure 1.** Photo of the Plant *C. depressus* worshipped in the festival “Jamajutia”, in Odisha,
14 India.

15

16 In the Indigenous system of medicine the above plant used as a cooling medicine in
17 fevers, as tonic; plants’ mucilage is prescribed in gonorrhoea. On stone, the roots are rubbed
18 and smeared over forehead to get relief in migraine; to cure leucorrhoea dried fruits are
19 powdered and taken orally with milk for 2 to 3 days. The plant is crushed with tender twigs of
20 *Prosopis cineraria*, mixed with whey and sugar and taken as a drink to treat body ache,

1 protrusion of uterus, urinal inflammation and to avoid abortion. Leaves are made into paste and
2 mixed with curd or whey and given orally to cure diarrhoea in children for 2-3 days [6].
3 It is also used to increase the viscosity of seminal fluid, to set-up menstrual disorder [7]. An
4 extract (specify which extract) of plant is used for its anti-diabetic activity and applied as a
5 paste in healing of wounds (The wealth of India) [8]. Ikram *et al* studied the hexane and
6 chloroform soluble whole plant extract of *C. depressus* which exhibited prominent antipyretic
7 activity in rabbits receiving subcutaneous yeast injections and it did not show any toxic or
8 adverse effect up to an oral dose of 1.6g/kg [9].
9 Pareek *et al* studied the *in vitro* hepatoprotective activity effect of *Corchorus depressus* L.
10 against CCl₄ induced toxicity in HepG₂ cell line. It was observed that the ethanolic extract
11 alleviated the changes induced by CCl₄ in a concentration dependent manner [10].
12 Kataria *et al* through their research demonstrated the *in vitro* and *in vivo* aphrodisiac properties
13 of *Corchorus depressus* Linn. on rabbit corpus cavernosum smooth muscle relaxation and
14 sexual behavior of normal male rats [11].
15 A survey of the published literatures revealed that the antioxidant activity of this plant has not
16 yet been subjected for scientific investigation. The aim of present research therefore is the
17 quantitative analysis of the phenolics and flavonoids content in different extracts (specify which
18 extract) of *Corchorus depressus* and study their antioxidant property by using different
19 protocols so as to authenticate the folkloric information about the utilization of this plant.

20 2. MATERIALS AND METHODS

21 2.1 Collection of Plant Material

22 *Corchorus depressus* Linn. (Tiliaceae) was collected from local area of Salipur at geographic
23 coordinates (latitude and longitude) and identified by Botanical Survey of India (BSI), Central

1 National Herbarium, Kolkata, INDIA (Authentication No- CNH/I-I/28/2009/Tech.II/93) and
2 (Figure 3&4)(?). A voucher specimen (SJCPS-T) was preserved in the herbarium of Sri
3 Jayadev College of Pharmaceutical Sciences (SJCPS), Naharkanta, Bhubaneswar, Odisha,
4 INDIA. ~~after its collection from local area of Salipur, present in state Odisha, India since (?)~~
5 ~~August 2009.~~

6 2.2 Chemicals

7 Standard quercetin, gallic acid, luteolin was obtained from Sigma Chemicals. All other
8 chemicals and reagent used were used from E-Merck and of analytical grade.

9 2.3 Experimental

10 2.3.1 Preparation of plant material

11 The plants were washed thoroughly under running tap water, chopped; air dried for a week at
12 35 to 40 °C. The leaves, stem and the root were separated to prepare the respective powders
13 separately. Each plant materials were pulverized in electric grinder separately to moderately
14 fine powders (355/180) [All particles pass through a No. 355 sieve and not more than 40%
15 through a No. 180 sieve] [12].

16 2.3.2 Extraction process

17 The moderately fine powder of the plant material (1 kg) was initially defatted with petroleum
18 ether. The defatting process concerning mainly with the elimination of chlorophyll (colouring
19 matter, wax in leaves or fixed oils and proteins) which may interfere with isolation of other
20 phyto-constituents from the extract. ← In this work, no compounds were isolated. Therefore, it
21 is a matter of obtaining an extract rich in constituents of a certain class of organic compound.
22 ← Suggestion: ... which may interfere in the phytoprospection of the extract under study. The
23 defatted material was processed to successive extraction employing solvents of different

1 polarity in ascending order. ← Suggestion: List the solvents used and the extract obtained after
2 recovery of the extracting solvent. And, if possible, the yield of the extract after drying. The
3 powder and the solvent were presented to maceration by stirring at each 4hrs interval for 48hrs
4 at room temperature ($25\pm 5^{\circ}\text{C}$) and then filtered with what man filter paper of 2 to 3 μm pore
5 size [13]. ← explain this procedure further. The procedure adopted was for shortening the
6 process of extraction, and to minimize the contact time of plant sample with solvent [14]. ← Is
7 this procedure better than exhaustive extraction?

8 2.3.3 Qualitative phytochemical analysis

9 In the present work, the qualitative phytochemical analysis was carried out in the different
10 extracts obtained from the selected plant. ← Specify what these extracts are. All the extracts
11 were subjected to various chemical tests as described in the different literatures (Wager, 1984;
12 Odebiyi, 1978; Trease and Evans 1987), for preliminary identification of various
13 phytoconstituents [15-17]. ← Suggestion: ...as described by Odebiyi, 1978; [15], Wager,
14 1984; [16] and Trease and Evans, 1987 [17], for preliminary identification of various classes
15 of phytoconstituents.

16 2.3.4 Total flavonoid content:

17 Aluminium chloride colorimetric method with a little modification was used to determine
18 the flavonoid content as per Chang *et.al.* 2002 [18]. Standard quercetin 10 mg dissolved in
19 96% ethanol and then it was used for preparation of calibration curve with different
20 concentrations like at 2, 4, 6, 8, 10 and 12 $\mu\text{g/ml}$. Quercetin solution (1 ml) and 1ml of extract
21 sample were mixed with 3 ml of 96% ethanol, 0.2ml of aluminium chloride 10%, 0.2 ml of
22 potassium acetate 1M and 5.6 ml distilled water. The mixture was incubated at room

1 temperature for 10 mins with occasional shaking. The absorbance was measured at 376 nm
2 against a blank without aluminium chloride using UV-Visible spectrophotometer.

3 **2.3.5 Determination of total phenolic content**

4 Folin-Ciocalteu's Reagent (FCR) method with **little modification** **Which one?** was used for
5 quantification of total **p**henolic compounds present in the **extract (?)** of *C. depressus*. In a 10 ml
6 volumetric flask, 0.2ml of plant extract and 0.5ml of Folin-Ciocalteu reagent (2N) was added.
7 After 3 minutes, 1ml of saturated sodium carbonate (20% in distilled water) was added in the
8 same volumetric flask. Final volume was made up to 10 ml with the distilled water. At 725nm
9 the absorbance of the blue coloured formed was measured after 1 hr against a distilled water
10 (blank) using UV-Visible spectrophotometer. A standard calibration curve with gallic acid was
11 plotted using different concentrations (Standard, 100-600 µg/ ml) [19, 20].

12 **2.3.6 Evaluation of *in-vitro* antioxidant activity**

13 The evaluation of the *in-vitro* antioxidant activity of *C. depressus* was carried out by using the
14 following methods [21];

15 **2.3.6.1 DPPH Radical scavenging activity**

16 The DPPH assay measured hydrogen atom (or one electron) donating activity and hence
17 provided an evaluation of anti-oxidant activity due to free radical scavenging. DPPH, a stable
18 and coloured (purple)-free radical which was reduced into the yellow-coloured **diphenyl-picryl**
19 **hydrazine**. **0.1mM solution of DPPH solution** 0.1mM in methanol was prepared. 1 ml of this
20 solution was mixed with 3ml of sample solutions in water at different concentrations. **Finally**
21 after 30 minutes of incubation period at room temperature, the absorbance was measured at
22 517 nm against the blank.

23 DPPH radical scavenging activity was calculated according to the following equation;

1
$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

2 being, A_0 the absorbance of the Control (without extract) and A_1 the absorbance of the sample.

3 **2.3.6.2 *In-vitro* hydroxyl radical scavenging activity**

4 The formation of hydroxyl radical (OH) from Fenton reagent was quantified using 2-
5 deoxyribose oxidative degradation. The principle of the assay is the quantification of the 2-
6 deoxyribose degradation product, malonaldehyde, by its condensation with thiobarbituric acid
7 (TBA). The reaction mixture contained deoxyribose (2.8 mM), FeCl₃ (100mM), KH₂PO₄-KOH
8 buffer (20mM, P^H 7.4), EDTA (100mM), H₂O₂ (1mM), ascorbic acid (100mM) and various
9 concentrations of the extracts in a final volume of 1 ml. Ferric chloride and EDTA (when
10 added) were remixed just before the addition to the reaction mixture. Reaction mixture was
11 incubated at 37⁰C for 30 mins. After incubation at 37⁰C for 30 mins, 1 ml of 2.8% trichloro
12 acetic acid and 1 ml of 1 % aqueous solution of TBA were added to the sample and the test
13 tubes were heated at 100⁰ C for 20 min to develop the colour. After cooling, TBARS formation
14 was measured spectrophotometrically (Perkin-Elmer) at 532 nm against an appropriate blank.
15 The hydroxyl radical scavenging activity was determined by comparing absorbance of the
16 control with that of test compounds. The hydroxyl radicals scavenging activity was calculated
17 according to the following equation;

18
$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

19 A_0 → Absorbance of the Control (without extract)

20 A_1 → Absorbance of the Sample.

21 See: 2.3.6.1

22 **2.3.6.3 *In-vitro* nitric oxide radical scavenging activity:**

1 Nitric oxide was generated from sodium nitroprusside, which at physiological P^H liberates
2 nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions which
3 diazotize with sulphanilic acid and couple with naphthylenediamine (Griess reagent),
4 producing pink colour which can be measured at 546 nm. Sodium nitroprusside (10 mM, 2 ml)
5 in phosphate buffer saline (P^H 7.4) was incubated with 0.5 ml of test compounds in different
6 concentrations at room temperature for 30 minutes. After 30 minutes, 0.5 ml of the incubated
7 solution was added with 1 ml of Griess reagent and the absorbance was measured at 546 nm.
8 The nitric oxide radical scavenging activity was calculated according to the following formula;

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

9
10 A_0 → Absorbance of the Control (without extract)

11 A_1 → Absorbance of the Sample.

12 See: 2.3.6.1

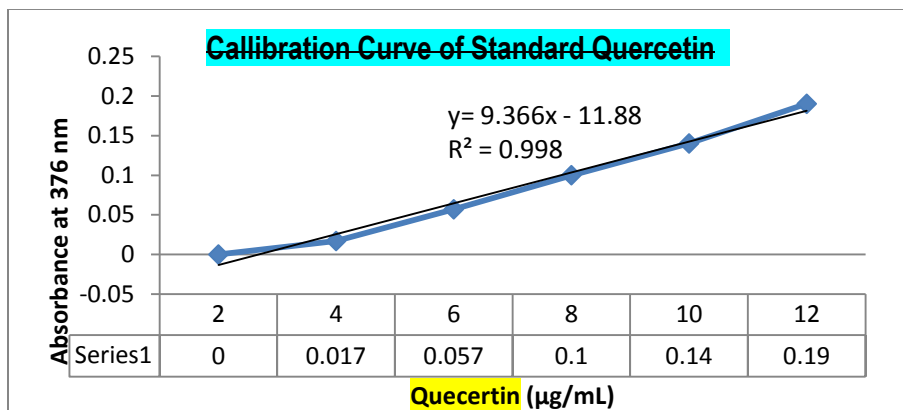
13 The same experiment was repeated with ascorbic acid, as taken in different concentrations for
14 methanolic and aqueous leaf extracts of *C. depressus*. ← Specify throughout the manuscript
15 that these were the extracts studied. And, that only the aqueous and methanolic extracts of the
16 leaves were studied. Water and methanol are solvents with close polarities. Think about it.

17 What about the stems and roots?

18 RESULTS AND DISCUSSION

19 Different extracts of *C. depressus*, were subjected to qualitative phytochemical analysis where
20 the methanolic and the aqueous leaf extracts displayed variety of classes of phytochemicals
21 present in the extract. The maximum (?) number of phytochemicals like the alkaloids,
22 glycosides, steroids, flavonoids, Saponins, tannins, and phenolic compounds were detected

1 from the methanolic leaf extract, followed by the detection of Saponins, flavonoids, tannins
2 and phenolic compounds from the aqueous extract. ← This sentence should be excluded!
3 With the measured absorbance data obtained spectrophotometrically, a standard calibration
4 curve was plotted for determination of total flavonoid content which was expressed as weight
5 of Quercetin equivalent (QE) at 100g extract (Figure 2).

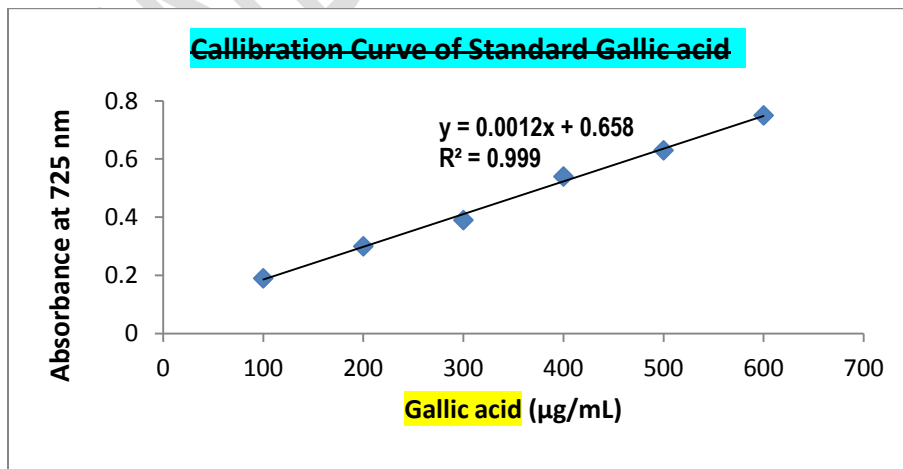


6
7 **Figure 2: Calibration curve of standard quercetin.**

8 → mg/ml, µg/ml already represents concentration.

9 The results obtained by FCR method, the total phenolic content of the extract was determined
10 as Gallic acid equivalents (GAE) from the plotted standard calibration curve of Gallic acid
11 (Figure 3).

12 **Figure 3: Calibration curve of Standard Gallic acid.**



13

1 See suggestion for Figure 2.

2 The results of total phenolic and flavonoid content of *Corchorus depressus* leaf extracts were
3 presented in (Table 1).

4

5 **Table 1. Total Phenolic and Flavonoid content of *Corchorus depressus* leaf extracts**
6 **expressed as (mg/100g) ← It is quoted in the body of the Table. ...in or as?**

Leaf Extracts of <i>C. depressus</i>	Total phenolics (mg/100g) in GAE	Total flavonoids (mg/100g) in QE
Aqueous	18.18	1.80
Methanolic	78.46	21.2
Petroleum ether	0.95	0.11
Chloroform	4.14	0.54
Ethyl acetate	7.2	0.99

7 Describe the process for obtaining these three extracts in the **Material and methods** section.

8 Left and right sides of the table should be open.

9

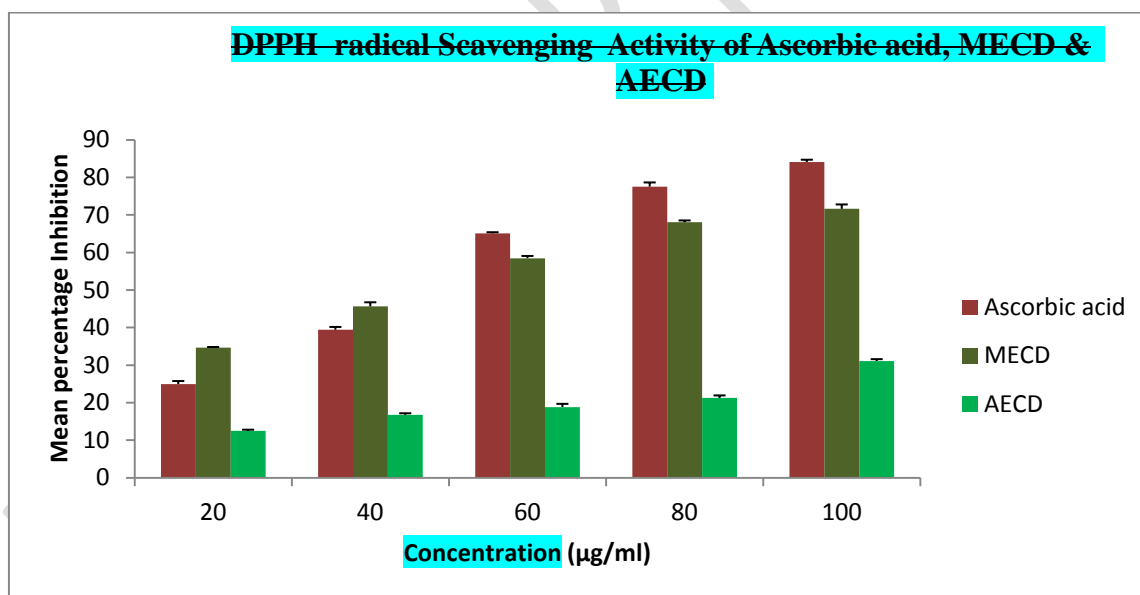
10 **The DPPH scavenging activity**

11 Anti-oxidant reacts with DPPH, which is a nitrogen-centered radical with a
12 characteristic absorption at 517 nm and convert it to 1, 1-diphenyl-2- picryl hydrazine, due to
13 its hydrogen donating ability at a very rapid rate. The degree of discoloration indicates the
14 scavenging potential of the anti-oxidant [22-23]. The extracts (?) and the standard ascorbic acid
15 were tested from lower to higher concentration (20-100 µg/ml). All the extracts & the ascorbic
16 acid exhibited DPPH radical scavenging activity in a concentration dependant manner. Among

1 the extracts, the methanolic leaf extract of *C. depressus* (MECD) showed a mean percentage
2 inhibition of (71.6%) at the concentration of 100µg/ml; whereas the mean percentage
3 inhibition for the aqueous leaf extract was found to be of 31.1% at 100 µg/ml. The results were
4 well compared to standard drug ascorbic acid, which showed the highest mean percentage of
5 inhibition (84.09%) at the concentration of 100µg/ml. A graph between the mean percentage
6 inhibitions of the DPPH radical scavenging activity of ascorbic acid, MECD & AECD were
7 presented as (Figure 4). ← The abbreviation MECD is in the item Abstract, but MECD and
8 AECD are not cited in Material and methods. Replace & by and. Make the necessary
9 adjustments throughout the text.

10

11 Figure 4. DPPH radical Scavenging Activity of Ascorbic acid, MECD & AECD.



12

13 See suggestion for Figure 2. Figures and Tables must be self-explanatory. Thus, suggestion for
14 Title: Figure 4. DPPH radical scavenging activity of ascorbic acid of aqueous (AECD) and
15 methanolic (MECD) extracts from *C. depressus* leaves.

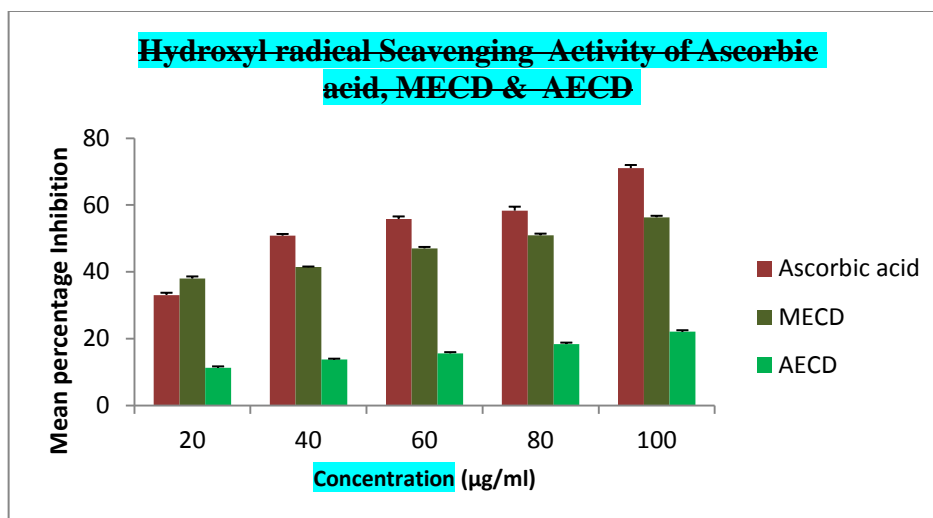
16

1

2 **Hydroxyl radical scavenging activity**

3 The test extracts MECD & AECD along with the standard ascorbic acid, suppressed hydroxyl
4 radical mediated deoxyribose degradation in a concentration dependant manner. The hydroxyl
5 radical is a highly potent oxidant that reacts with almost all biomolecules found in the living
6 cells [24]. When it reacts with poly unsaturated fatty acid moieties of cell membrane
7 phospholipids, lipid hydroperoxide is produced. Lipid hydroperoxide can be decomposed to
8 alkoxy and peroxy radical and numerous carbonyl products such as malondialdehyde (MDA).
9 ← Acronym used only here. The carbonyl products are responsible for DNA damage,
10 generation of cancer, respiratory and aging related diseases [25, 26]. The hydroxyl radical
11 scavenging activity of the methanolic leaf extract of *C. depressus* (MECD) showed 56.31% at
12 100µg/ml, which is higher in comparison to the aqueous extract (AECD) which showed 22.5 %
13 at 100µg/ml. However the ascorbic acid showed the highest mean percentage of hydroxyl
14 radical scavenging activity of 71.04% at 100µg/ml. A graph showing the mean percentage
15 inhibition of hydroxyl radical scavenging activity of the standard Ascorbic acid, the MECD &
16 AECD was depicted in (Figure 5).

17 **Figure 5. Evaluation of Hydroxyl radical scavenging activity of Ascorbic acid, MECD**
18 **and AECD.**

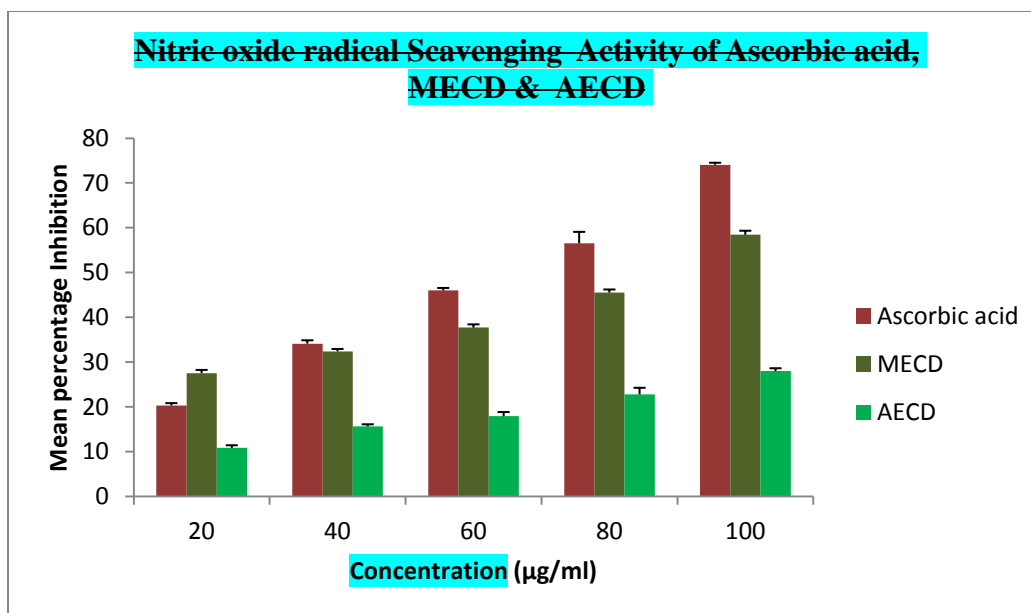


See suggestion for Figure 4.

Nitric oxide scavenging assay:

Nitric oxide (NO) is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems, including neuronal messenger, vasodilation, anti-microbial and anti-tumor activities [27, 28]. Studies in animal models have suggested a role for NO in the pathogenesis of inflammation and pain. NOS inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue changes seen in models of inflammatory bowel disease [29]. The mean percentage inhibition of the nitric oxide scavenging activity of methanolic leaf extract of *C. depressus* was 58.44 % at the concentration of 100µg/ml. This value was higher in comparison to the mean percentage inhibition of aqueous leaf extract (AECD) of the same plant which showed 27.99 % at 100µg/ml. However the standard ascorbic acid showed highest mean percentage inhibition (74.07 %) at the concentration of 100 µg/ml (Figure 6).

Figure 6. Nitric oxide radical scavenging activity of ascorbic acid, MECD and AECD.



See suggestion for Figure 4.

3. CONCLUSION

Normally free radicals of different forms are generated at a low level in cells to help in modulation of several physiological functions and are quenched by an integrated anti-oxidant system in the body. However if the free radicals are produced in excess amount, they can be destructive, leading to generation of various types of diseases including diabetes, causing delay in the healing of wound and its repair. ← This sentence can be transferred to the Results and Discussion item. Therefore in the current study, different extracts of *C. depressus* were studied for their possible anti-oxidant activity by DPPH radical, hydroxyl & nitrous oxide scavenging activity at different concentrations. Among all, the methanolic leaf extract of *C. depressus* (MECD) showed better anti-oxidant activity in comparison to the aqueous extract ← In all concentrations? The activity increased in a dose-dependent manner. This result may be due to the presence of saponins and flavonoids in the methanolic extract. Thus the methanolic leaf

1 extract of the plant have the potency for treating oxidative stress during different diseases and
2 may be responsible for different biological activity attributed to *C. depressus*.

3

4 **CONSENT**

5 All the authors have given their consent for submission of the manuscript to the esteemed
6 Journal.

7 **ETHICAL APPROVAL**

8 It is not applicable for this article.

9

10 **COMPETING INTERESTS DISCLAIMER:**

11

12 Authors have declared that no competing interests exist. The products used for this research
13 are commonly and predominantly use products in our area of research and country. There is
14 absolutely no conflict of interest between the authors and producers of the products because
15 we do not intend to use these products as an avenue for any litigation but for the
16 advancement of knowledge. Also, the research was not funded by the producing company
17 rather it was funded by personal efforts of the authors.

18

19 Please check and standardize all references following the JPRI Guidelines for authors

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