Original Research Article

Free-radical Scavenging and Quantitative Estimation of Flavonoids from the polar extracts of *Corchorus depressus* leaves

ABSTRACT

Background: The investigation of total flavonoids and 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 diphenyl picryl hydrazine, hydroxyl radical and nitric oxide radical scavenging methods were performed for measurement of the antioxidant activity at different extracts. The flavonoid and phenolic content of the extracts were determined by using aluminium chloride and Folin-Ciocalteau'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.

Conclusion: The methanolic extract of *C. depressus* at 100µg/ml showed highest DPPH, hydroxyl and nitric oxide radical scavenging activity and this activity may be attributed to the presence of saponins and flavonoids as detected in the extract.

Key words: Antioxidant, Flavonoids, *Corchorus depressus,* Phenolic content

1. INTRODUCTION

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

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

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

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

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

Figure 1. Photo of the Plant *C.depressus* worshipped in the festival "Jamajutia", in Odisha, India.

In the Indigenous system of medicine the above plant used as a cooling medicine in fevers, as tonic; plants' mucilage is prescribed in gonorrhoea. On stone, the roots are rubbed and smeared over forehead to get relief in migraine; to cure leucorrhoea dried fruits are powered and taken orally with milk for 2 to 3 days. The plant is crushed with tender twigs of *Prosopis cineraria*, mixed with whey and sugar and taken as a drink to treat body ache, protrusion of uterus, urinal inflammation and to avoid abortion. Leaves are made into paste and mixed with curd or whey and given orally to cure diarrhoea in children for 2-3 days [6].

It is also used to increase the viscosity of seminal fluid, to set-up menstrual disorder [7]. The plant is used for its anti-diabetic activity and applied as a paste in healing of wounds [8]. Ikram *et al* studied the hexane and chloroform soluble whole plant extract of *C. depressus* which exhibited prominent antipyretic activity in rabbits receiving subcutaneous yeast injections and it did not show any toxic or adverse effect up to an oral dose of 1.6g/kg [9].

Pareek *et al* studied the *invitro* effect of C*orchorus depressus* L. against CCl_4 induced toxicity in $HepG_2$ cell line. It was observed that the ethanolic extract alleviated the changes induced by CCl_4 in a concentration dependent manner [10].

Kataria *et al* through their research demonstrated the *invitro* and *invivo* aphrodisiac properties of *Corchorus depressus* Linn. on rabbit corpus cavernosum smooth muscle relaxation and sexual behavior of normal male rats [11].

A survey of the published literatures revealed that the antioxidant activity of this plant has not yet been subjected for scientific investigation. The aim of present research therefore is the quantative analysis of the phenolics and flavonoids content in methanolic and aqueous extracts of *Corchorus depressus* and study their antioxidant property by using different protocols so as to authenticate the folkloric information about the utilization of this plant.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

Corchorus depressus Linn. (Tiliaceae) was collected from local area of Salipur at geographic coordinates (20.4843^0 N, 86.1192^0 E) and identified by Botanical Survey of India (BSI), Central National Herbarium, Kolkata, INDIA (Authentification No- CNH/I-I/28/2009/Tech.II/93). A voucher specimen (SJCPS-T) was preserved in the herbarium of Sri Jayadev College of Pharmaceutical Sciences (SJCPS), Naharkanta, Bhubaneswar, Odisha, INDIA

2.2 Chemicals

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

2.3 Experimental

2.3.1 Preparation of plant material

The plants were washed thoroughly under running tap water, chopped; air dried for a week at 35 to 40 ^{0}C. The leaves, stem and the root were separated to prepare the respective powders. However the powder of leaves were used in this case for experimental purpose. Each plant materials were pulverized in electric grinder separately to moderately fine powders (355/180) [All particles pass through a No. 355 sieve and not more than 40% through a No. 180 sieve] [12].

2.3.2 Extraction process

The moderately fine powder of the leaves (1 kg) was initially defatted with petroleum ether. The defatting process concerning mainly with the elimination of chlorophyll (colouring matter, wax in leaves or fixed oils) which may interfere in the phytoprospection of the extract under study. The defatted material was processed to successive extraction employing solvents of different polarity in ascending order (e.g. chloroform, ethyl acetate, methanol and aqueous). The powder and the solvent was presented to maceration by stirring at each 4hrs interval for 48hrs at room temperature (25±5^{0}C) and then filtered with what man filter paper of 2 to 3 µm pore size [13]. The successive extractive values of the petroleum ether, chloroform, ethyl acetate, methanolic and aqueous leaf extracts are 1.22%, 2.05%, 1.85%, 12.15% and 9.0% respectively. The procedure adopted (maceration under agitation) was for shortening the process of extraction, and to minimize the contact time of plant sample with solvent. Besides, the extraction at ambient temperature was also a compromise between extraction efficiency and limitation of thermal alteration of extracted bio molecules hence maceration under agitation was better in comparison to exhaustive extraction [14]. The methanolic and aqueous leaf extracts of *Corchorus depressus* were denoted as MECD and AECD respectively.

2.3.3 Qualitative phytochemical analysis

The qualitative phytochemical analysis was carried out in the methanolic and aqueous extracts obtained from the selected plant. Both the extracts were subjected to various chemical tests as described by Wager, 1984; [15], Odebiyi, 1978;[16], Trease and Evans, 1987[17] for preliminary identification of various classes of phytoconstitutents.

2.3.4 Total flavonoid content

Aluminium chloride colorimetric method with a little modification was used to determine the flavonoid content as per Chang *et.al.* 2002 [18]. Standard quercetin 10 mg dissolved in 96% ethanol and then it was used for preparation of calibration curve at 2,4,6,8,10 & 12 μg/ml. Quercetin solution (1 ml) and 1ml of extract sample were mixed with 3 ml of 96% ethanol, 0.2ml of aluminium chloride 10%, 0.2 ml of potassium acetate 1M and 5.6 ml distilled water. The mixture was incubated at room temperature for 10 mins with occasional shaking. The absorbance was measured at 376 nm against a blank without aluminium chloride using UV-Visible spectrophotometer.

2.3.5 Determination of Total Phenolic Content

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

2.3.6 Evaluation of *In-vitro* Antioxidant Activity

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

2.3.6.1 DPPH Radical scavenging activity

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

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

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

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

2.3.6.2 *In-vitro* hydroxyl radical scavenging activity

The formation of hydroxyl radical (OH) from Fenton reagent was quantified using 2-deoxyribose oxidative degradation. The principle of the assay is the quantification of the 2-deoxyribose degradation product, malonaldehyde, by its condensation with thiobarbituric acid (TBA). The reaction mixture contained deoxyribose (2.8 mM), $FeCl_3$ (100mM), KH_2PO_4-KOH buffer (20mM, pH 7.4), EDTA (100mM), H_2O_2 (1mM), ascorbic acid (100mM) and various concentrations of the extracts in a final volume of 1 ml. Ferric chloride and EDTA (when added) were remixed just before the addition to the reaction mixture. Reaction mixture was incubated at 37^0C for 30 mins. After incubation at 37^0C for 30 mins, 1 ml of 2.8% trichloro acetic acid and 1 ml of 1 % aqueous solution of TBA were added to the sample and the test tubes were heated at 100^0 C for 20 min to develop the colour. After cooling, TBARS formation was measured spectrophotometrically (Perkin-Elmer) at 532 nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing absorbance of the control with that of test compounds. The hydroxyl radicals scavenging activity was calculated according to the following equation;

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

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

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

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

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

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

The same experiment was repeated with ascorbic acid, as taken in different concentrations for methanolic and aqueous leaf extracts of *Corchorus depressus*.

3 RESULTS AND DISCUSSION

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.

The extracts of *Corchorus depressus*, were subjected to qualitative phytochemical analysis where the methanolic and the aqueous leaf extracts displayed classes of phytochemicals presence in the extract. The phytochemicals like the alkaloids, glycosides, steroids, flavonoids, saponins, tannins, and phenolic compounds were detected from the methanolic leaf extract, followed by the detection of saponins, flavonoids, tannins and phenolic compounds from the aqueous extract.

With the measured absorbance data obtained spectrophotometrically, a standard callibration curve was plotted for determination of total flavonoid content which was expressed as weight of quercetin equivalent (QE) at 100g extract **(Figure 2)**.

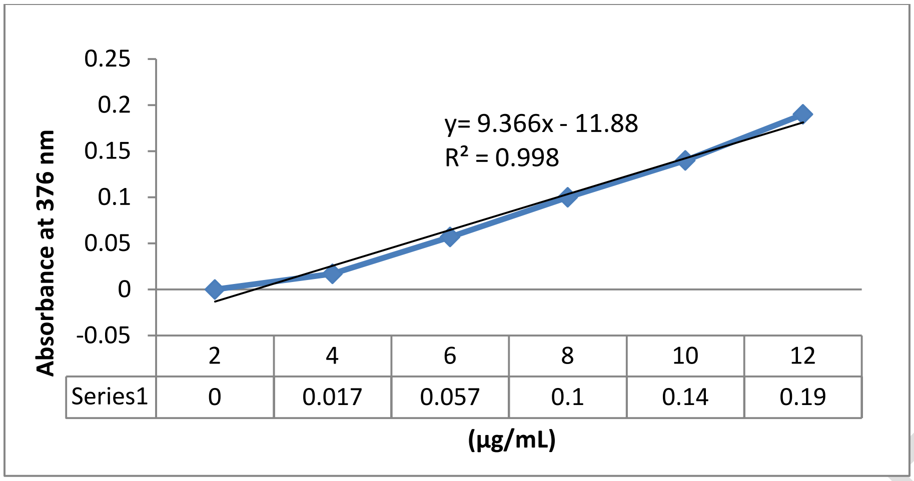

Figure 2: Callibration curve of standard quercetin.

The results obtained by FCR method, the total phenolic content of the extract was determined as gallic acid equivalents (GAE) from the plotted standard callibration curve of gallic acid **(Figure 3)**.

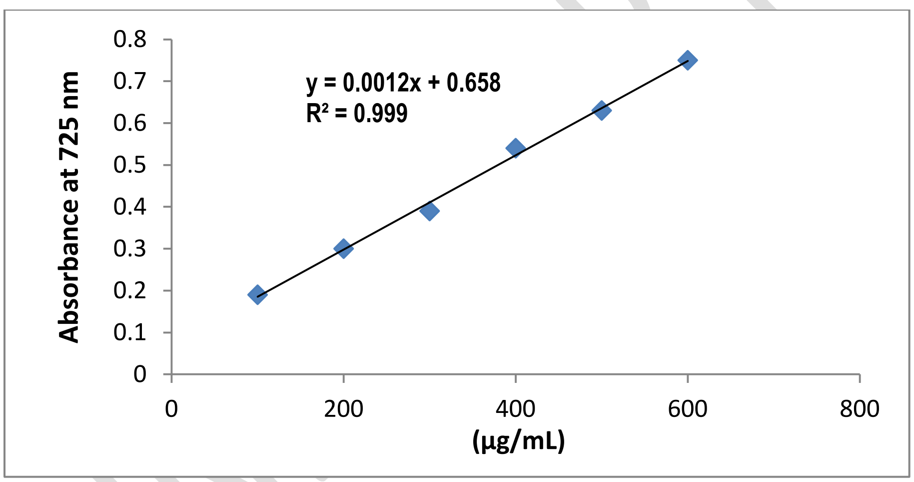

Figure 3: Callibration curve of standard gallic acid.

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

Table 1. Total Phenolic and Flavonoid content of *Corchorus depressus* leaf extracts

Leaf Extracts of *C. depressus*	Total phenolics (mg/100g) as GAE	Total flavonoids (mg/100g) as 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

The DPPH scavenging activity

Anti-oxidant reacts with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and convert it to diphenyl picryl hydrazine, due to its hydrogen donating ability at a very rapid rate. The degree of discoloration indicates the scavenging potential of the anti-oxidant [22-23]. The extracts (methanolic and aqueous) and the standard ascorbic acid were tested from lower to higher concentration (20-100 µg/ml). Both the extracts and the ascorbic acid exhibited DPPH radical scavenging activity in a concentration dependant manner. Among the extracts, the methanolic leaf extract of *Corchorus depressus* (MECD) showed a mean percentage inhibition of (71.6%) at the concentration of 100µg/ml; whereas the mean percentage inhibition for the aqueous leaf extract was found to be of 31.1% at 100 µg/ml. The results were well compared to standard drug ascorbic acid, which showed the highest mean percentage of inhibition (84.09%) at the concentration of 100µg/ml. A graph between the mean percentage inhibitions of the DPPH radical scavenging activity of ascorbic acid, MECD and AECD were presented as **(Figure 4).**

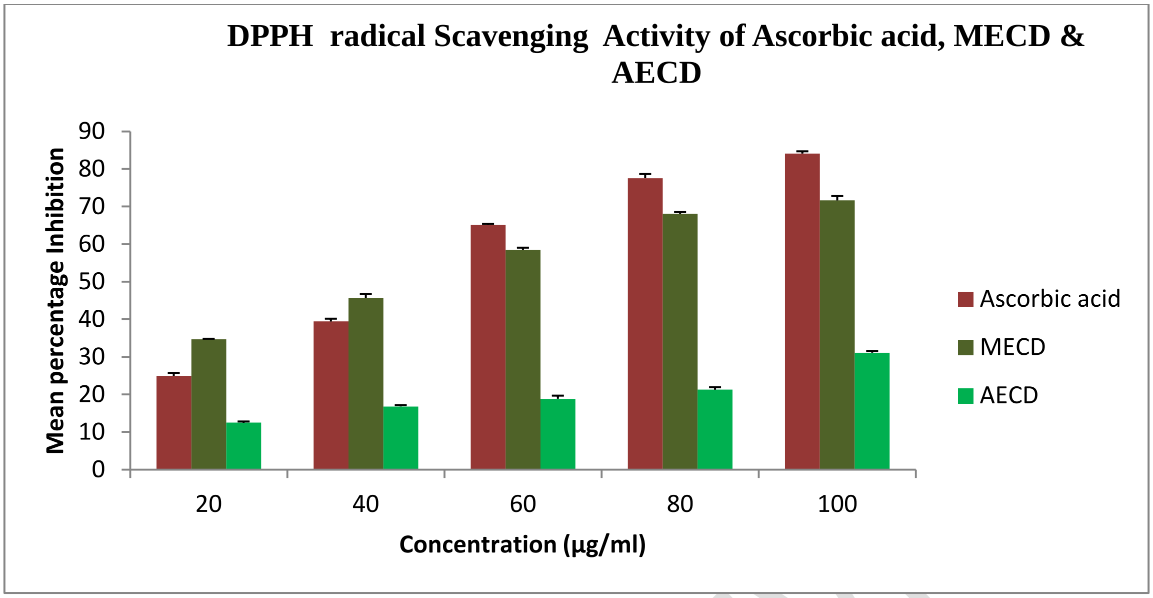

Figure 4. DPPH radical scavenging activity of ascorbic acid, methanolic (MECD) and aqueous (AECD) extracts from *Corchorus depressus* leaves.

Hydroxyl radical scavenging activity

The test extracts MECD and AECD along with the standard ascorbic acid, suppressed hydroxyl radical mediated deoxyribose degradation in a concentration dependant manner. The hydroxyl radical is a highly potent oxidant that reacts with almost all biomolecules found in the living cells [24]. When it reacts with poly unsaturated fatty acid moieties of cell membrane phospholipids, lipid hydroperoxide is produced. Lipid hydroperoxide can be decomposed to alkoxyl and peroxyl radical and numerous carbonyl products such as malondialdehyde. The carbonyl products are responsible for DNA damage, generation of cancer, respiratory and aging related diseases [25, 26]. The hydroxyl radical scavenging activity of the methanolic leaf extract of *C. depressus* (MECD) showed 56.31% at 100µg/ml, which is higher in comparison to the aqueous extract (AECD) which showed 22.5 % at 100µg/ml. However the ascorbic acid showed the highest mean percentage of hydroxyl radical scavenging activity of 71.04% at

100µg/ml. A graph showing the mean percentage inhibition of hydroxyl radical scavenging activity of the standard Ascorbic acid, the MECD and AECD was depicted in (**Figure 5)**.

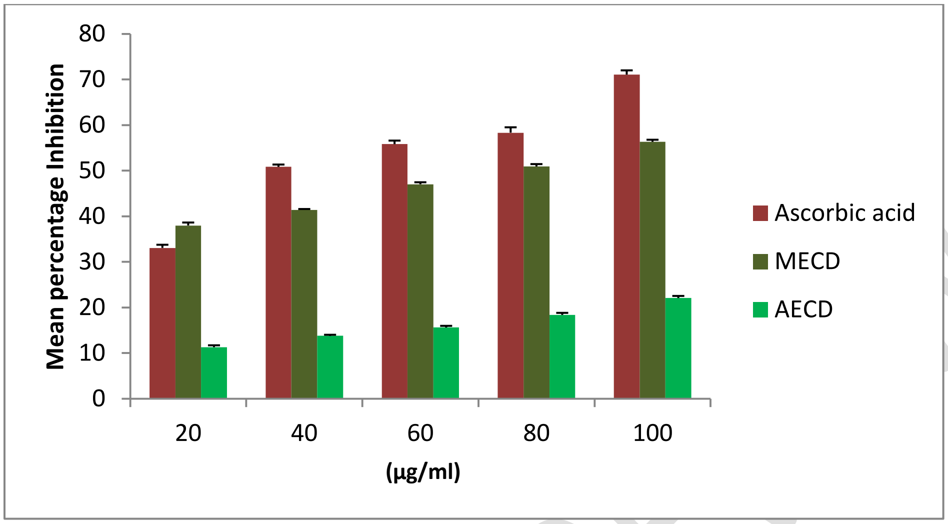

Figure 5. Evaluation of hydroxyl radical scavenging activity of ascorbic acid, methanolic (MECD) and aqueous (AECD) extracts from *Corchorus depressus* leaves.

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**).

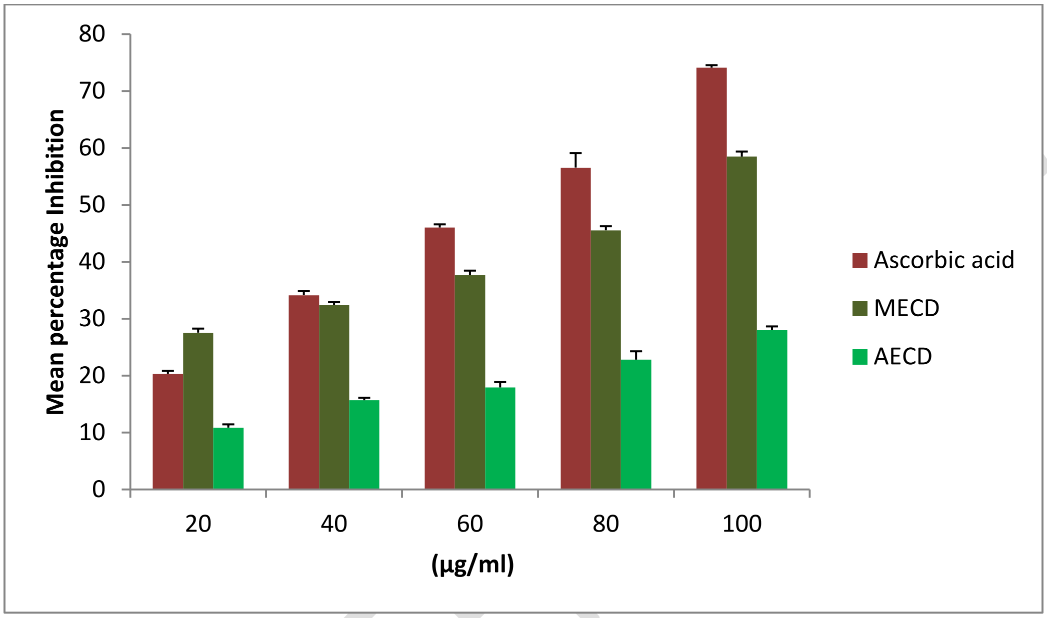

Figure 6. Nitric oxide radical scavenging activity of ascorbic acid, methanolic (MECD) and aqueous (AECD) extracts from *Corchorus depressus* leaves.

4 CONCLUSION

In the current study, methanolic and aqueous extracts of *C. depressus* were studied for their possible anti-oxidant activity by DPPH radical, hydroxyl & nitrous oxide scavenging activity. The methanolic leaf extract of *C. depressus* (MECD) showed better anti-oxidant activity in comparison to the aqueous extract in all concentrations and 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 extract of the plant have the potency for treating oxidative stress and may be responsible for different biological activity attributed to *C. depressus*.

CONSENT

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

ETHICAL APPROVAL

It is not applicable for this article.

COMPETING INTERESTS DISCLAIMER

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

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