International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 08-04-2019; Accepted: 11-05-2019
www.botanyjournals.com
Volume 4; Issue 4; July 2019; Page No. 25-28

Antioxidant, Cytotoxicity activities and phytochemical analysis of *Chenopodium murale* (Linn.)

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Abstract
Chenopodium murale Linn. is an evergreen annuals herb, and can be found throughout the year. *Chenopodium murale* are thought to possess therapeutic effects on many illnesses in worldwide. Present study was aimed to investigate an antioxidant, cytotoxic activities and phytochemical analysis of *Chenopodium murale* ethanol extract. The antioxidant activities of the extracts of *Chenopodium murale* were determined with methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity, hydrogen peroxide (H_2O_2) and superoxide (NBT) radical scavenging activity, determination of total phenolic content and total flavonoid contents. The binding action of the DPPH, ABTS, H_2O_2 and NBT radicals scavenging activity increases with concentration of the extracts. Methanol extract exhibited higher cytotoxic activity against brine shrimp. The total phenolic and flavonoid content estimated was 500 mg/g Gallic acid equivalent and 19160 mg/g rutin equivalent respectively. Results of the present study suggest that *Chenopodium murale* extract can be an alternative to synthetic antioxidant, anticancer and antibiotic agents.

Keywords: *Chenopodium murale*, DPPH, ABTS, NBT, H_2O_2, scavenging activity, cytotoxicity total phenolic, flavonoid compounds

1. Introduction
Chenopodium murale is a member of Chenopodiaceae and is used traditionally for prevention of liver disorders. It has several medicinal properties reported like: antibacterial, antifungal, anti-diaphoretic, anti-asthmatic, migraine, digestive problems, sterility, anxiolytic, antidepressant and anti-hypertensive etc. Its leaves decoction is used in the treatment of jaundice (Ahmad, 2003, Jan, 2009).
In traditional medicines more than 20,000 plants species are used, and all of these bioactive fractions for development of new drugs. In industrialized countries advance medicine research replaced the medicinal plant because the large number of world population cannot afford pharmaceutical drugs and use indigenous medicines prepared from plants. Therefore, traditionally used medicinal plants have received comparatively more attention because from their bioactive constituents new drugs are prepared (Tabuti, 2003) [20].
The oxidative processes in the human body result the production of various free radicals. The excess of these free radicals cause cellular damage that results different human diseases such as diabetes mellitus, cancer and others inflammatory disorder (Wang *et al*., 2005; Tiwari and Tripathi, 2007) [23, 21]. Antioxidants found in the medicinal plants are the only hope in getting rid of these harmful molecules. Polyphenols are present in the plant raw material having significant antioxidant properties (Prior *et al*., 2005) [16]. Therefore attention has given to medicinal plants for their active role in prevention of human diseases due to presence of anti-oxidative phytochemical (Vinary *et al*., 2004).
During the past decade, many therapeutic agents of cancer induce a process of cell death known as apoptosis, or programmed cell death. Although the killing tumors mechanisms through apoptotic path ways by the chemotherapy processes have been controversial, but the influence of apoptosis has been now used as a novel method for anticancer drugs identification (Mulholland, 2005, Elvin- Lewis, 2001, National Acad Sci, 1989) [13, 6, 4]. Medicinal plant possesses bioactive non-nutrients compounds are called phytochemicals that have properties to decrease the major chronic diseases risk. Several thousands of phytochemicals have been investigated in fruits, vegetables and grains but still greater numbers of these are unknown and there is a need to investigate (Liu, 2003) [12]. Flavonoids have therapeutic effects and are due to properties of antioxidant. In addition to properties of antioxidant flavonoid compounds protect from the heart disease due to inhibitory effects of lipoxygenase and cyclooxygenase activities, macrophages and platelets (Hollman, & Arts, 2000). With this work, we aimed to contribute to the understanding and discovery of powerful antioxidants for safe use of this herb for treatment of different ailments. In short our result suggests that this medicinal plant can be used for treatment of ailment in which free radical damages are involved. The cytotoxicity findings suggest that it can be used as anticancer or as antibiotic. The objectives of this research were to determine antioxidant and anticancer activities of the extract of *Chenopodium murale.*

2. Materials and Methods
The plant of *Chenopodium murale* was collected from the vicinity of District Bannu, identified by Dr. Sultan Mahmood. The specimen of *Chenopodium murale* (Herbarium No: 2012-04) is kept at the herbarium of the Botany Department, Bannu University. The collected whole plants were washed, dried in shed and then changed to powder with electric grinder. Plant powder was soaked in 70% methanol and shake randomly for 10 days. The extract was filtered through Whatman filter paper No. 1 and residues were evaporated (40°C) with rotary evaporator.

2.1 Phytochemical Analysis Methods

Preliminary phytochemical Analysis of *Chenopodium murale* extract for various phytoconstituents such as phenols, flavonoid, tannins, saponins, terpenoids, phlobatannins, cardiac glycoside, cumarins, and anthraquinones and steroids was carried out according to the modified methods of Khandelwal (2007) [8].

Total flavonoid content of the extract was performed by the method of Kumaran and Karunakaran, (2006) [11]. The amount of total flavonoid compounds was calculated as µg Rutin (RE)/g *C. murale* by using an equation that has been obtained from the rutin calibration curve.

Total phenolic contents of C. *murale* extract was identified accordingly with the procedure of Zhinshen *et al*. (1999) [24]. The quantity of the total phenolic compounds was denoted as µg gallic acid equivalent (GAE)/g *C. murale*.

2.2 Cytotoxic method

Cytotoxic potential of the extract of *C. murale* was estimated using Brine shrimps according to the method of Meyer-Albert *et al*., (1992).

2.3 Antioxidant Activity methods

Total antioxidant capacity of the extract was estimated by DPPH (1, 1, biphenyl, 2-picrylhydrazyl) test (Hassan *et al*. 2000) [7]. Antioxidant capacity was clarified as µg ascorbic acid equivalent (AAE)/g. Hydrogen peroxide radical scavenging assay was carried out by the method of of Ruchet *et al*. (1989). Ascorbic acid used as standard antioxidant agents. Nitroblue-tetrazolium (NBT) radical scavenging activity of *C. murale* extract was established by the reduction method of (Nishikini *et al*. 1972). Ascorbic acid was performed as standard antioxidant agent. ABTS test was employed by the method of Re *et al*. (1999). Ascorbic acid was performed as standard antioxidant agent.

DPPH radical scavenging activity, ABTS radical scavenging activity, H_2O_2 radical scavenging activity and NBT radical scavenging activities were calculated using the following equation:

$$\% \text{ inhibition} = (Ac - As / Ac) \times 100$$

A_C is the absorbance of the control, As is the absorbance of the sample.

For all antioxidant, and cytotoxicity test, three replicates of each extract were used and the values are expressed as Mean ± Sd. (Duncan, *et al*., 1977).

3. Results and Discussion

Preliminary qualitative analysis of methanol extract was performed and different phytochemical present in *Chenopodium murale* methanol extract were tannins, saponins, cardiac glycosides, terpenoids, cumarins, flavonoid, anthraquinones and phenolic compounds (Table-1). The total flavonoid was 191.60g/gm Rutin equivalent and total phenolic compound was 0.5g/gm Gallic acid equivalent. The result of the anti-oxidant activity and reducing power activity of methanol extract are due to phenolic compounds as present in this species and there is a positive co-relation between the flavonoid, phenolic and antioxidant activity (Rice-Evans *et al*., 1996) [18].

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of antioxidant compounds in plant. The *C. murale* (49.93%) DPPH free radical scavenging activity is less potent in compared with the ascorbic acid (65.76%) at 250µg/ml presented in Fig-1. The results revealed potent hydroxyl radical scavenging activity of plant extract (49.91%) as indicated by the obtained IC_{50} values of plant extract at 250µg/ml (fig- 2). Hydrogen peroxide though itself is not very reactive but some may lead to cytotoxicity due to generation of hydroxyl radicals in the cells. It has also been reported that iron complexes occurring inside the cell react with hydrogen peroxide in-vivo and highly reactive hydroxyl radicals generate which attribute to toxic effects, (Kumar and Hemalatha, 2011) [10]. The percentage NBT Superoxide scavenging capacity of the extract is 49.70% at 250µg/ml. The result depicted potent plant extract scavenging activity as seen through the obtained IC_{50} value (fig-3). The biochemical mechanisms for oxygen toxicity include lipid per-oxidation and superoxide radical generation. Superoxide is harmful and damage to the tissue by producing various diseases. It is a precursor of the more reactive species which cause oxidative stress (Aderogba, 2005) [1]. This superoxide radical can propagate or inhibit the process of lipid per-oxidation. The free radical scavenging activity of the test plant along with reference standards, such as ascorbic acid were determined by ABTS assay and the results are presented in (Fig- 4). The ABTS radical activity is applicable for both hydrophilic and lipophilic antioxidants. The extract had potent (50%) antioxidant abilities that near the control, Vitamin C (65.76%) at 250µg/ml. The scavenging activity of the extracts also could be due to the presence of higher levels of flavonoid compounds which may have contributed to their high antioxidant activity as reported by Afroz *et al.,* (2006) [2]. The overall results depicted plant extract potential antioxidant activity and may be attributed to the presence of high quality phenols, flavonoid and tannins. These phyto-constituents are strong reducing agents, singlet oxygen quenchers and hydrogen donors that contribute in minimizing the oxidative stress by the scavenging action, due to the presence hydroxyl groups (Morton *et al*., 2012) [14]. DPPH, ABTS, NBT and H_2O_2 radicals scavenging activities are less effective than the commercial available synthetic like Ascorbic acid. It can be said that the scavenging effects on the DPPH, ABTS NBT and H_2O_2 radicals activity strongly dependent on the extract concentration. Our results suggesting a potential antioxidant activity of the plant extract of *Chenopodium murale* and can be used for treatment of ailment in which free radicals are implicated. Preliminary free radical scavenging activity provides helpful information about the therapeutic value of this plant extract. The findings obtained by Duenas *et al.,* (2006) [5] and Kilani *et al.,* (2008) [9] also support our results.

Under controlled condition cytotoxic effect of *Chenopodium murale* extract was evaluated against brine shrimps survival. The effect of extract was found that the concentration of the plant extract is inversely proportional to the brine shrimps survival. The IC_{50} value of the plant extract was 80µg/ml (Table-2). Results of *Chenopodium murale* extract also showed cytotoxic activity might be due to presence of bioactive constituents. So it can be used for the treatment of cancer or as antibiotic. Cytotoxicity screening model provides initial data to select extracts of plant with neoplastic properties for future work (Cardellina *et al*., 1999) [3].

Table 1: Composition of both extracts of *Chenopodium murale* (CM)

Constituents	CM extract	AM leaves extract
Flavonoid	+	+
Tannin	+	+
Saponins	+	+
C.glycoside	+	+
Steroids	_	_
Cumarins	+	+
Terpenoids	+	+
Anthraquinones	+	+
Phlobatannins	+	+

'+' represents presence and, _, represents absence

Table: 2: Cytotoxicity brine shrimps assay of extract of *Chenopodium murale*.

Treatment	%age inhibition of extract of CM against brine shrimps cytotoxicity after 24 hrs				IC_{50}
	50 µg/ml	80 µg/ml	100 µg/ml	120 µg/ml	
Chenopodium murale	30%	50%	70%	100%	80 µg/ml

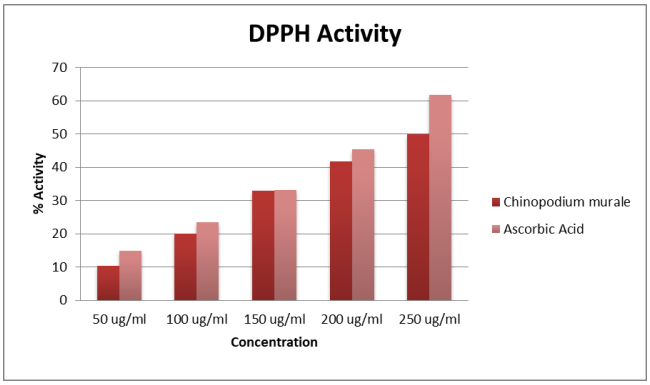

Fig 1: DPPH scavenging activity of methanol extract of *Chenopodium murale* compared with standard Ascorbic acid

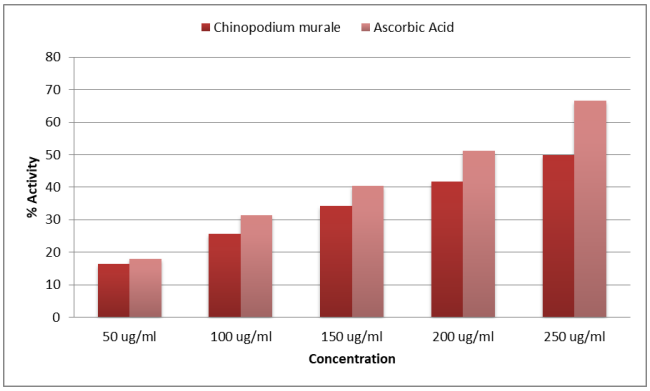

Fig 2: Hydrogen peroxide scavenging activity of methanol extract of *Chenopodium murale* compared with standard ascorbic acid

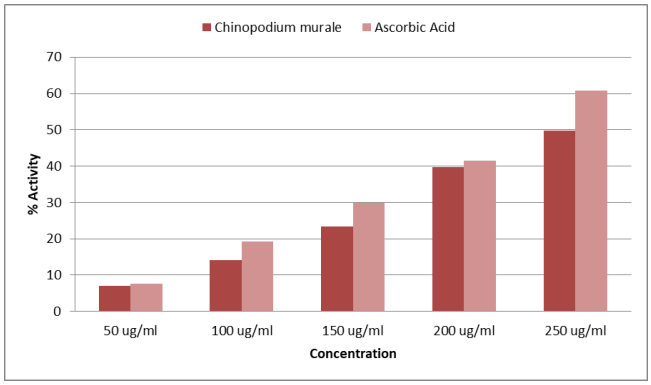

Fig 3: Superoxide radical scavenging activity of methanol extract of *Chenopodium murale* compared with a standard Ascorbic acid

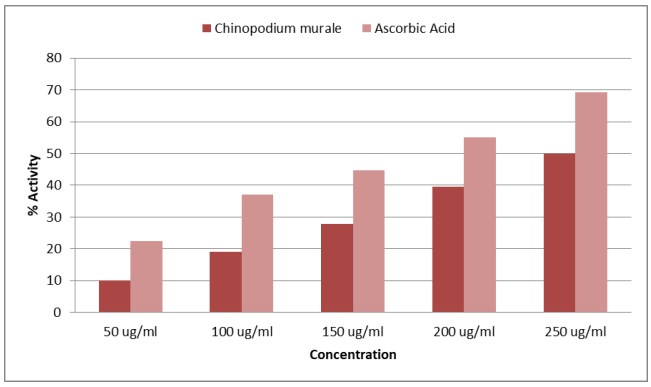

Fig 4: ABTS scavenging activity of methanol extract of *Chenopodium murale* in comparison with standard Ascorbic acid.

4. Conclusion

The results of *Chenopodium murale* showed potent antioxidant activity by inhibiting, DPPH, Superoxide radical and also have potent cytotoxic activity. In addition plant extract contain high amount of flavonoid and small amount of total phenolic compound which play important role in controlling oxidation. The methanol extract showed potent anti-oxidative activities against DPPH, Hydrogen peroxide, ABTS, and NBT radicals. The results suggest that the phenolic compounds present in this extract are promising source of natural products that can be developed as anti-oxidant agents and can be used as anti-cancer or as antibiotic.

5. References

1. Aderogba MA, Okoh EK, Idowu TO. Evaluation of the antioxidant activity of secondary metabolites from Pilostiga reticulata DC. Hoechst. J. Biol. Sci. 2005; 5:239-242.
2. Afroz Alamgir S, Khan M, Jabbar MTH, Nahar N. Choudhuri S. Antidiarrhoeal activity of the ethanol

extracts of Paederia foetida Linn. (Rubiaceae). Journal of Ethnopharmacology. 2006; 105:125-130.
3. Cardellina JH, Fuller RW, Gable WR, Westergaard C, Boswell J, Munro MHG. Evolving strategies for the selection, dereplication andprioritization of antitumor and HIV-inhibitory natural products extract. In: Bohlin, L., 1999.
4. Bruhn JG. (Eds.), Bioassaay Methods in *Natural Product Research* and Development.Kluwer Academics Publishers, Dordrecht, pp. 25-36.
5. Duenas M, Hernandez T, Estrella I. Assessment of in-vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chem*. 2006; 98:95-103.
6. Elvin- Lewis M. Should we be concerned about herbal remedies? J of Ethnopharmacol. 2001; 75:141-164.
7. Hassan MS, Ahmed MI, Mondale S, Uddin SJ, Masud MM, Sadhu SK, *et al*. Antioxidant anticiceptive activity and general toxicity study of Dendrophthoe falcate and isolation of quercitin as the major component, Orient. Pharm. and Experi. Medic. 2000; 6:355-360.
8. Khandelwal KR. Practical pharmacognocy Techniques and Experiments' 17st Edition, Pune, India; Nirali Prakashan, 2007, 149-156.
9. Kilani S, Sghaier MB, Limem I, Boubakar J, Bhouri W, Skandrani I, Neffati A, *et al*. In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubersinfusion and extracts of Cyperus rotundus. Bioresource Tech. 2008; 99:9004-9008.
10. Kumaran A, Hemalatha S. In-vitro antioxidant activity of alcohol leaf extract and subfractions of Alangium Lamarckii Twaites. J. Chem. Pharm. Res. 3 suppl. 2011; 1:259-267.
11. Kumaran AJ, Karunakaran. In-vitro antioxidant activities of methanol extract of five phyllanthus species from India. LWT–Food. Sci. Techno. 2006; 40:344-352.
12. Liu RH. Health benefits of fruits and vegetables are from additive synergistic combination of phytochemicals. Am. J. Clin. Nutr. 2003; 78:517S-520S.
13. Mulholland DA, Chukwujekwu JC, Smith P, Coombes PH. Antispasmodial diterpenoid from leaves of Hyptis suaveoleus. J. of ethanopharmacol. 2005; 102:295-297.
14. Morton LW, Abu R, Amsha Caccetta IB, Puddey KD. Croft Chemistry and biological effects of dietary phenolic compounds, relevance to cardiovascular disease. Clin. Exp. Pharm. Phys. 2000; 27:152-159.
15. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazinemetho-sulphate and molecular oxygen. Biochem. and Biophys. Res. communica. 1972; 46:849-854.
16. Prior RL, Wu X, Schaech K. Standardized methods for the determination of antioxidant capacity and phenolic in foods and dietary supplements. J. Agri. Food. Chem. 2005; 53:4290-4302.
17. Re R, Pellegrino N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cat-ion decolonization assay. Free. Radic. Biol. Med. 1999; 26:123-1237.
18. Rice-Evans C, Miller N, Penganga G. Structure, antioxidant activity relationship of flavonoids and phenolic acids. Free. Radic. Biol. Med. 1996; 20:033-956.
19. Ruche RJ, Cheng SJ, Claug JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea, carcinogen. 1989; 10:1003-1008.
20. Tabuti JRS, lye KA, Dhillon SS. Traditional herbal drugs of Bulamogi, Uganda plants, use and administration. J. Ethnopharmacol. 2003; 88(1):19-44. http://dx. doi. org/10. 1016/S0378-8741(03)00161-2
21. Tiwari OP, Tripathi YB. Antioxidant properties of different fractions of vitex negundo Linn. Food. Chem. 2007;. 100:1170 -1176.
22. Vinery BP, *et al*. Antioxidant activity of some selected medicinal plants in Western region of India, 1996.
23. Wang K, Zhang Y, Yang C. Antioxidant phenolic compounds from rhizomes of polygonum paleaceum. J. Ethnopharm. 2005; 96:483-487.
24. Zhinshen J, Mengcheng T, Jianming W. The determination of flavonoid content in mulberry and their scavenging effects in superoxide radicals. Food. Chem. 1999; 64:555-9.