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Antioxidant activity and phytochemical screening of some common weeds available in Patna Women's College campus

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Abstract : *The phytochemical screening and the antioxidant activity of the methanolic and aqueous extracts of three weeds Phyllanthus niruri , Basella alba and Amaranthus tricolor were investigated and they revealed the presence of phenols, flavanoids, tannins, alkaloids, terpenoids and steroids. Of all three weeds selected, Phyllanthus niruri showed less H_2O_2 % inhibition as compared to Basella alba and Amaranthus tricolor. But the aqueous extract showed fairly moderate inhibition of 29.64% near to standard. Amaranthus tricolor showed highest % inhibition in all three weeds, methanolic and aqueous extracts showed 31% and 32%, respectively. Basella alba also showed % inhibition of 29% and 28% in aqueous and methanolic extracts, respectively. The Basella extract demonstrated highest total antioxidant capacity as flavanoid was detected in the extract. In P. niruri, methanolic extract showed higher activity than aqueous extract, however, in A. tricolor results were just the opposite. Plants exhibited reducing power ranging from 0.981 to 4.091. Amaranthus tricolor showed highest absorbance i.e high reducing power as compared to Phyllanthus niruri and Basella alba. The aqueous extract in Basella alba showed high reducing power as compared to the methanolic extract. However, in Phyllanthus niruri, methanolic extract showed high reducing power as compared to the aqueous extract. The antioxidant property is concentration dependent. There is variability in the antioxidant activity in the weeds. But all three weeds showed fairly moderate antioxidant activity. The results obtained in the study indicated that weed extracts are a potential source of natural antioxidants.*

Keywords : *Antioxidant activity, free radicals, hydrogen peroxide, phenol, flavanoids.*

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Introduction :

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as, catalase, superoxide dismutase and various peroxidases. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases (Mandal *et. al.*, 2009).

Plants develop several antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O_2 formed due to exposure to ultraviolet radiation. Our daily diet contains vegetables, fruits, tea, juices, etc. which possess compounds rich in anti oxidative properties (Kumar *et. al.*, 2008).

The free radicals play a very important role in human health and are beneficial in combating several diseases. During a chemical reaction (oxidation), one reactant loses an electron and is called oxidant or free radical, while the other gains an electron. In living organisms oxygen in unstable form is the most common free radical. This is called Reactive Oxygen Species (ROS) and is generated during various metabolic activities. Contaminants in the environment as well as normal metabolism of a cell, can change molecule into a free radical. The examples of ROS are OH, O_2 , H_2O_2, O_3, HOCI, RO_2, RO. Any molecule can become a free radical by either losing or gaining an electron. Once initiated these free radicals get involved in chain reaction with stable types. The compounds thus formed have longer stability in body and increase the potential for cellular damage. Free radicals damage the cell at the site of their operation causing serious disorders. Plaque may accumulate in arteries on oxidation. LDL Cholesterol functions as free radical and damages the free artery lining. It hampers the blood circulation which may lead to heart attack. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last three decades (Aruoma, 1998).

There is dynamic balance between amounts of free radicals generated in the body and antioxidant to scavenge them and prevent body from their deleterious effect. The cause of disease condition like hypertension, diabetes, etc are preliminary disorders due to imbalance between prooxidant and antioxidant homeostasis (Gulcin *et. al.,* 2004).

Plant derived natural products such as, flavanoids, terpenoids, and steroids *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. This has attracted a great deal of research interest in natural antioxidant. The most commonly used synthetic antioxidants presently are butylated hydoxyanisole (BHA), butylatedhydoxytoluene (BHT) Propylgallate (PG) and test butylated hydroquinone. However, these synthetic antioxidants have side effects such as, liver damage and carcinogenesis. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicine to replace synthetic antioxidant. (Kumar *et. al.,* 2008).

The present investigation aims to study weeds for their anti oxidant property together with their phytochemical screening.

Materials and Methods :

Collection of plant material: The weeds selected for antioxidant activity, were collected from the campus of Patna Women's College, Patna and were identified by Dr. Tanuja (Assistant Professor, Department of Botany, B.M.D. college, BRABU) as *Phyllanthus niruri*, *Basella alba* and *Amaranthus tricolor* and were maintained in the herbarium sheets for reference with voucher specimen no. as 1/01, 2/01 and 3/01 for *Phyllanthus niruri*, *Basella alba* and *Amaranthus tricolor,* respectively.

Sample preparation and extraction: Plant materials air dried under the shade for two weeks were powdered in a grinder and stored at room temperature. From each of the weeds, 5 g of plant material was extracted with 250 ml each (1:50, w/v) of solvent A (99% methanol) and solvent B (Distilled water). For methanolic extracts dried powder samples were extracted in a Soxhlet apparatus with methnol until becoming colorless. The extracts were filtered through Whatman No. 1 filter paper. The filtrates were centrifuged (REMI CENTRIFUGE, CODE:R8C-BL) at 1500 rpm for 20 minutes to remove any plant debris. For aqueous extract 5 g of powdered plant material of each weed was extracted by soaking in 250ml of water for 2 days in glass containers at room temperature followed by filtration with Whatman No. 1 filter paper. Filtrates were centrifuged at 1500 rpm for 20 minutes. Supernatants were stored at 22^0C until assayed.

Phytochemical screening of weeds : The methanolic extract and the aqueous extract of all three weeds were subjected to preliminary phytochemical testing for the detection of major chemical groups. **Test for Tannins:** 2-3ml of the methanolic and the aqueous extracts of each weed were taken in test tubes separately. Then 1ml of alcoholic ferric chloride ($FeCl_3$) was added in each test tube. Dark blue or greenish grey coloration of the solution indicates the presence of tannins in the sample **Test for Flavanoids:**2-3ml of the methanolic and the aqueous extracts of weeds were taken separately in test tubes. A few pieces of magnesium turnings with 1ml of conc. HCl were added to the extracts Pink red or red coloration of the solution indicates the presence of flavanoids in the sample. **Test for Alkaloids:**3ml of the methanolic and the aqueous extracts of weeds were taken separately in test tubes with the Wanger's reagent which includes 1.27gm of Iodine(I), 2gm potassium iodide(KI) and 100ml of DW. Brown precipitate indicates the presence of alkaloid in the sample.**Test for phenols:** The methanolic and the aqueous extracts of weeds were spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spots and was exposed to ammonia vapours Blue coloration of the spot indicates the presence of phenols. **Test for steroids and terpenoids:**To 1 ml of extract of weed, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. The test was done for all the three weed samples extracted Dark green coloration of the solution indicates the presence of Steroids and dark pink or red coloration of the solution indicate the presence of terpenoids.

Hydrogen peroxide scavenging activity: Ability of the extracts to scavenge hydrogen peroxide was determined as described by Govindarajan *et al*. (2003) and Gulcin *et al*. (2004). One ml of the extract was rapidly mixed with 2 ml of 10 mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer (THERMO SCIENTIFIC, after 10 min of incubation at 37^oC against a blank (without hydrogen peroxide). The percentage of inhibition of hydrogen peroxide was calculated using the following formula.

% inhibition H_2O_2 = ([A0]-[A1])/ [A0] X 100

Where, (A0 – Absorbance of control; A1 – Absorbance of sample)

Total antioxidant capacity : The total antioxidant capacity of the crude extracts of plant materials was evaluated by the method of Prieto *et. al*. (1999). The antioxidant capacity of the extracts was measured spectrophotometrically using phosphomolybdenum method, based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate / Mo(V) compounds. A 0.3 ml aliquot of the sample solution was combined with 2.7 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All samples were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. For the blank, 0.3 ml ethanol was mixed with 2.7 ml of the reagent. A typical blank solution contained 2.7 ml of reagent solution and the appropriate volume of Methanol was used for the dissolution of the samples and it was incubated under the same conditions as the rest of the samples.

Reducing power assay: The reducing power of the extracts was determined according to the method of Oyaizu (1986). All weed extracts and standard antioxidants in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (10 g/l). The mixtures were incubated at 50°C for 20 min. Then, 2.5 ml of TCA (10%; 2.5 ml) was added to each mixture and centrifuged at 3000 rpm for 20 min. Finally, the supernatants (2.5 ml) were mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml; 0.1%). The absorbance of the solutions was measured at 700 nm. A higher absorbance of the reaction mixture indicated that the reducing power had increased.

Results and Discussion :

Phytochemical screening: Phytochemical screening revealed the presence of steroids, flavanoids, alkaloids, terpenoids, tannins and phenols in weeds selected (Table1). The flavanoids, as an antioxidant, may contribute to the effects such as, hepatoprotective and nephroprotective, antimicrobial, anti-inflammatory, and anti-carcinogenic. Alkaloids have anti-malariae, analgesic properties and also useful in the in treatment of stomach disorder. This is consistent with the past works of Okwu et. al. (2005). Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects (Okwu and Morah, 2004). Tannins have astringent properties, and they hasten the healing of wounds and inflamed mucous membranes (Okwu et. al., 2005). These phytochemicals ultimately contribute to the antioxidant property of weeds. Depending upon the concentration of these phytochemicals, the antioxidant property of weeds varies.

Table 1: Phytochemiacal Screening of weeds

NAME OF THE WEED	TANN-INS	FLAVA-NOIDS	ALKAL-OIDS	STER-OIDS	TERPE-NOIDS	PHEN OLS
1. ***Phyllnathus niruri***						
a) Aqueous extract	+	–	+	–	+	+
b) Methanolic extract	+	+	+	–	+	+
2. ***Basella alba***						
a) Aqueous extract	+	+	–	+	–	+
b) Methanolic extract	+	+	–	+	–	+
3. ***Amaranthus tricolor***						
a) Aqueous Extract	+	–	+	+	+	+
b) Methanolic Extract	+	–	+	+	+	+

+ :- indicates presence, – :- indicates absence

In *Phyllanthus niruri* all phytochemicals except steroid were present in methnolic extract, but in aqueous extract flavanoid and steroid were not detected. In *Basella alba,* tannins, flavanoid, steroid and phenol were present but alkaloid and terpenoid were not detected. In *Amaranthus tricolor* all phytochemicals except flavanoid, were present.

Hydrogen peroxide scavenging activity of Weeds*:* The measurement of H_20_2 scavenging activity is one of the useful methods of determining

the ability of antioxidants to decrease the level of pro-oxidants such as, H_2O_2. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells. The inhibitive effect of weeds extract were subjected to hydrogen peroxide scavenging assay and were found to be moderate when compared to ascorbic acid taken as standard.

Of all three weeds selected *Phyllanthus niruri* showed less % inhibition as compared to *Basella alba* and *Amaranthus tricolor*. But the aqueous extract showed fairly moderate inhibition of 29.64% near to standard. *Amaranthus tricolor* showed highest % inhibition in all three weeds, in both methanol and aqueous extracts i.e. of 31% and 32%, respectively. *Basella alba* also showed % inhibition of 29% and 28% in aqueous and methanolic extracts, respectively.

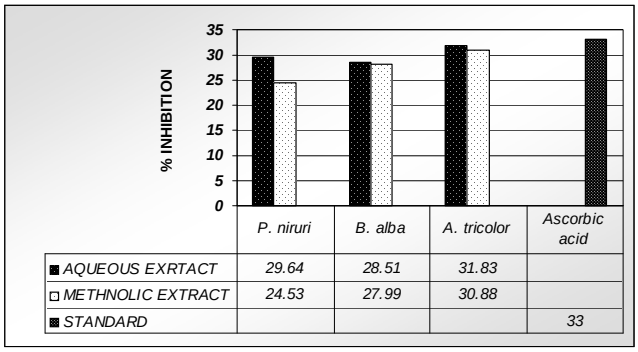

Figure1: H_2O_2 Scavenging activity of Weeds

Total antioxidant capacity: The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, a-tocopherol, and carotenoids (Prieto *et. al*., 1999). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols, and aromatic amines have the ability to donate hydrogen and electrons. In general, aqueous alcohol is considered the best solvent for extracting phenolic compounds from plant materials (Negi *et. al*., 2003). The antioxidant activities of weed extracts were measured by the phosphomolybdenum method. It can be seen that different vegetables exhibited various degrees of antioxidant activity. All weed extracts showed antioxidant activity in the phosphomolybdenum method. The results presented above indicated that the antioxidant activity of weeds seems to be due to the presence of polyphenols, flavanoid and anthocyanoside that may act by donating electrons and free radicals. Flavanoids have been shown to have potent antioxidant activity. The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products. The antioxidants break the free radical chain by donating a hydrogen atom (Gordon, 1990; Dorman *et. al.*, 2003).

The results presented above indicated that there is a potential anti oxidant activity in all three weeds studied. Flavanoids have been shown to have potent antioxidant activity (Ng *et. al*., 2000). The *Basella* extract demonstrated highest total antioxidant capacity as flavanoid has been detected in the extract. In *P. niruri*, methanolic extract, showed higher activity than aqueous extract, however, in *A. tricolor* results were just the opposite, as shown in Figure 2.

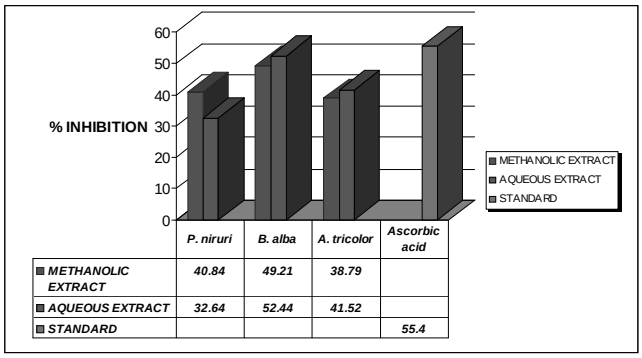

Figure 2: Total antioxidant capacity of weed as compared to standard

Reducing power assay : The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et. al*., 1995). Reducing potential is generally associated with the presence of reductants such as antioxidant substances, which cause a reduction in the Fe^{3+}/ferricyanide complex to Fe^{2+}. Accordingly, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. An electron-donating reducing material deals with the antioxidant activity to donate an electron to free radicals from the solution. The reducing power capacities of the samples were compared to ascorbic acid. Plants exhibited reducing power ranging from 0.981 to 4.091. All of the three weeds showed more or less significant reducing power at the same level as standard antioxidant, because of some degree of electron donation capacity in a concentration dependent manner. Extracts containing the highest amounts of total phenolics had weaker reducing power than compounds although results were close. Similar relationship between Fe^{3+} reducing activity and total phenol content have been reported in the literature (Goa *et. al*., 2000).

Amaranthus tricolor showed highest absorbance i.e high reducing power as compared to *Phyllanthus niruri* and *Basella alba*. In *Basella alba* aqueous extract showed high reducing power as compared to the methanolic extract. However in *Phyllanthus niruri*, methanolic extract showed high reducing power as compared to the aqueous extract as shown in Figure 3. These variations may be due to the different concentration of phenol in the extract. Further, investigation is needed to confirm these variations.

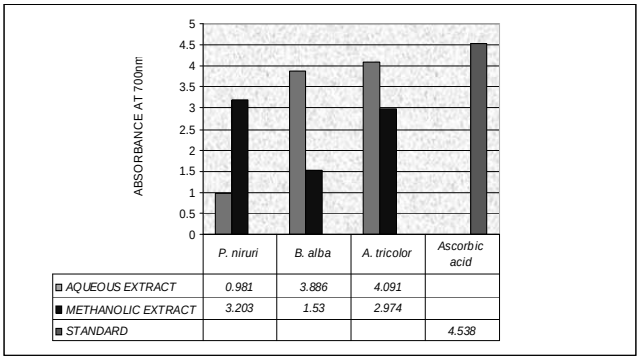

Figure 3: Reducing power assay

Conclusion :

On the basis of the results of this study, it is concluded that the aqueous and methanolic extracts of all three weeds i.e. *Phyllanthus niruri* , *Basella alba* and *Amaranthus tricolor* have significant antioxidant activity compared to other well characterized, standard antioxidant such as, Ascorbic acid in vitro. In addition, the antioxidant activity may be due to the presence of phytochemicals such as, steroids, flavanoids, alkaloids, terpenoids, tannins and phenols in weed extracts.

The antioxidant property is concentration dependent. There is variability in the antioxidant activity in the weeds. But all three weeds showed fairly moderate antioxidant activity. The results obtained in the study indicated weed extracts as potential source of natural antioxidants.

However, the components responsible for the antioxidative activity of weeds are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components of weeds.

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