

A Preliminary Study on Phytochemical Screening of *Boerhaavia Diffusa*, *Euphorbia Hirta* and *Amaranthus Polygonoides*

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ABSTRACT

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The objective of the present study was to evaluate the phytochemical constitution and antioxidant activity of Aqueous extracts of three selected plant *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides*. Preliminary phytochemical screening revealed the presence of phytochemicals like alkaloids, flavonoids, Steroids, phenols, tannin and carbohydrates in *Boerhaavia diffusa* and *Euphorbia hirta* where as in *Amaranthus polygonoides* many phytoconstituents like alkaloids, flavonoids, Steroids, terpenoids, phenols, saponin, tannin and carbohydrates were present. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. The free radicals (oxidants) are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. The results of antioxidant activity of three aqueous extract showed maximum activity in different concentration of 50, 250, 500, 750 and 1000 µg/ml. The percent inhibition of aqueous extract of *Boerhaavia diffusa*, *Euphorbia hirta*, *Amaranthus polygonoides* was 176.15, 404.78 and 413.06% respectively. In the present work potent anti-oxidant activity of aqueous extract of *Boerhaavia diffusa* was higher when compared to other two extracts. The present study revealed that the plant extract possessed good antioxidant activity and less quantity of toxic metals, which therefore can be used as a natural source of free radical scavenger. However, further study needs to be carried out to know its mode of action.

KEYWORDS: Phytochemical screening, antioxidant activity, DPPH, *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides*

INTRODUCTION

Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.*, 2000). The importance of plants is well known. The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson and Wright, 1996), anti-hepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar and Muthuselvam, 2009). Medicinal plants contain some organic compounds which have a definite physiological action on the human body. These bioactive substances include tannins,

alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edogaet *et al.*, 1978).

Phytochemical originating from plant sources are nothing but bioactive compounds which are also known as secondary metabolites. There are two types of metabolites produced in plants viz. Primary metabolites and Secondary metabolites. During the past several years, phytochemicals have been used worldwide as traditional herbal medicine. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Ugochukwu *et al.*, 2013) The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009)

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing biomolecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc (Halliwell and Gutteridge, 1984; Maxwell, 1995). Antioxidants are the compounds which terminate the attack

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of free radicals and thus reduce the risk of these disorders (Rice-Evans et al., 1996). Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione (Prior and Cao, 1999). Keeping this in view, the present study has been conducted to evaluate the comparative phytochemical and antioxidant activity of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* which are traditionally well known for their high activity.

Materials and Methods

Collection of Plant Materials

The fresh samples of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* were collected randomly from the Tiruvannamalai Dt. Tamil Nadu. Sample materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in a refrigerator.

Preparation of Extracts

Crude Sample extract was prepared by Soxhlet extraction method. About 20gm of powdered sample material was uniformly packed into a thimble and extracted with 250ml of aqueous extract separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor becomes colourless. After that the extract was taken in a beaker kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C till future use.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for all the extracts of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test for the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Detection of Flavonoids

H₂SO₄ test: Extracts were treated with few drops of H₂SO₄. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

Liebermann- Burchard test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H₂SO₄. The colour changed from violet to blue or green in some samples indicates the presence of steroids.

Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% NH₃ were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

Detection of Phenols

Ferric chloride test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of froth (appearance of creamy stable persistent small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

DPPH scavenging activity assay

Inhibition of diphenyl-2-picrylhydrazyl (DPPH) radicals by the tested plant extracts were measured according to a method reported by Molyneux (2004), with minor modifications. A mixture consisting of an extract solution at different concentrations (50 µL) and the methanolic solution of the DPPH reagent (50 µL) was loaded into each well of a 96-well plate. The mixture was left to stand for 30 minutes in the dark, and then the absorption was measured at 517 nm using the Nanodrop apparatus. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = (Ac-As/Ac) × 100; where RSA: radical scavenging activity; Ac: absorbance of the negative control; As: absorbance of the plant sample or ascorbic acid.

Result and Discussion

The phytochemical and antioxidant properties of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* have been evaluated by preliminary studies and by measuring their DPPH radical scavenging assay using crude aqueous extract of these plants.

Preliminary phytochemical screening

Phytochemicals are the core of phytomedicines; their therapeutic efficiency directly correlates with the presence of various phytochemicals (Somit et al., 2014). The screening of the aqueous extracts of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides*, phytochemical

constituents was performed using generally accepted techniques for qualitative determination. The study indicated that alkaloids, flavonoids, Steroids, phenols, tannin and carbohydrates were present in all studied plant extracts, while Terpenoids and tannins were present only in *Amaranthus polygonoides*. The phytochemical characteristics were summarized in the (Table 1). Identification of plant chemical constituents is desirable because such information will be value for synthesis of complex chemical substances.

Plants are known to contain a wide variety of secondary metabolites. These secondary metabolites or bioactive compounds generate definite physiological actions on the human system. According to (Yadav et al., 2011) around 25 percent of all prescribed medicines today are substances derived from plants. Phenols when mixed with the flavonoid compounds in plants are reported to show multiple activities like antioxidant, anticarcinogenic, anti-inflammatory etc [Asha et al., 2011].

Table 1: Preliminary phytochemical screening of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides*

Phytochemicals	<i>Boerhaavia diffusa</i>	<i>Euphorbia hirta</i>	<i>Amaranthus polygonoides</i>
Alkaloids			
Mayer's test	+	+	+
Wagner's test			
Flavonoids			
Lead acetate test	+	+-	+
H ₂ SO ₄ test			
Steroids			
Liebermann-Burchard test	+	+	+-
Terpenoids			
Salkowski test	-	-	+-
Arthroquinone			
Borntrager's test	-	-	-
Phenols			
Ferric chloride test	++	++	+-
Lead acetate test			
Saponin	-	-	++
Tannin	+	+	+
Carbohydrates	++	+	++
Oils & Resins	-	-	-

Antioxidant Activity

DPPH Assay

Antioxidant is compounds that delay or inhibit the oxidative damage. The most likely mechanism of antioxidant protection is direct interaction of the extract (or) compounds and the hydrogen peroxide rather than altering the cell membranes and limiting damage (Annan and Houghton, 2008). The DPPH assay is based on the assumption that an antioxidant serves as a hydrogen donor and thus reduces (decolorizes) DPPH free radicals (the color turns from purple to yellow). This assay is well-known as a basic, quick tool to evaluate antioxidant activity of putative antioxidants. Thus, the antioxidant potency of a compound is relative to the loss of DPPH free radicals (DPPH scavenging) that can be quantified through a decrease in the maximum absorption of DPPH at 570 nm. In this study, results showed that all samples had significant levels of radical scavenging activity in a dose dependent manner. The DPPH-derived IC₅₀ values of the plant extracts are also illustrated in Figure.

The DPPH assay for aqueous extract of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* was studied at different concentrations. The extract showed maximum activity in different concentration of 50, 250, 500, 750 and 1000 µg/ml. The aqueous extract showed 40-67% of scavenging activity. The Inhibition concentrations were 176.15 µg/ml, 404.78 µg/ml and 413.06µg/ml for *Boerhaavia diffusa*, *Euphorbia hirta* , *Amaranthus polygonoides* respectively.

Figure 1: DPPH assay for aqueous extract of *Boerhaavia diffusa*

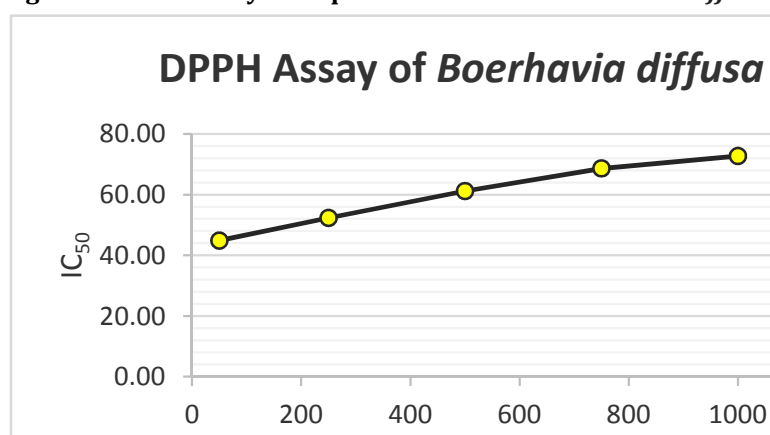
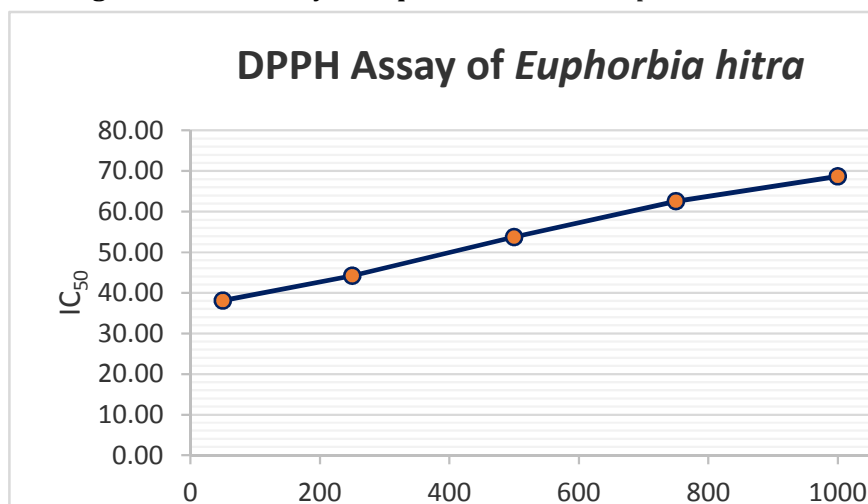
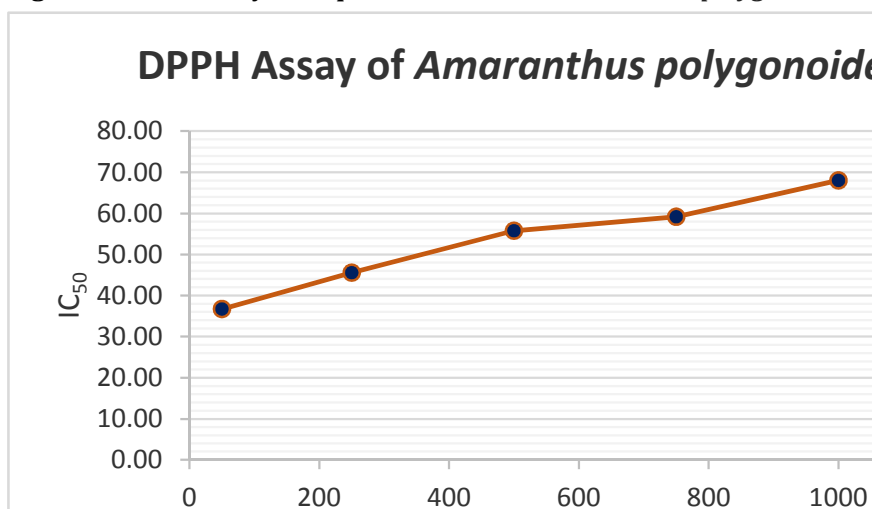


Figure 2: DPPH assay for aqueous extract of *Euphorbia hirta***Figure 3: DPPH assay for aqueous extract of *Amaranthus polygonoides***

Conclusion

In this study, the qualitative phytochemical analysis and the antioxidant properties of Aqueous leaf extracts of *Boerhaavia diffusa*, *Euphorbia hirta* and *Amaranthus polygonoides* were evaluated. The results obtained may suggest that the aqueous extract of the three plants possess compounds with antioxidant properties which can be used as natural preservative for food or cosmetic products. The present study reveals that extracts have good source of antioxidant property contain phyto-constituents. The presence of alkaloids, flavonoids, Steroid, phenols, tannin and carbohydrates in the extracts may be responsible for their antioxidant activity. From this study it is observed that all the plants possess marked antioxidant effect. These antioxidant properties are responsible for the various pharmacological activities.

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