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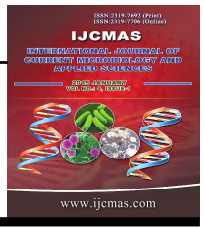

Original Research Article

DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India

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Keywords

Antioxidant, DPPH, Flavonoids, Medicinal Plants, Phenolics, and Phytochemical

A B S T R A C T

Methanolic extracts of *Gymnema sylvestre* (leaf), *Holarrhena antidysenterica* (bark), *Vernonia anthelmintica*(seeds) *Enicostemma littorale* (leaf), *Momordica charantia* (fruit), *Swertia chirata* (leaf), *Azadirachta indica* (leaf), *Caesalpinia bonducella* (leaf) used in Ayurvedic medicines for number of ailments were evaluated for their antioxidant activity.The free radical-scavenging activity of the extracts was measured as decolourizing activity followed by the trapping of the unpaired electron by1, 1-diphenyl-2-picryl hydrazyl radical (DPPH).The percentage decrease of DPPH was recorded maximum in *A. indica* followed by *M. charantia, C. bonducella, E.littorale, V. anthelmintica, S.chirata, H.antidysenterica, G.sylvestre*. The antioxidant activity of medicinal plants was at par with the commercial antioxidant like L-Ascorbic acid. Phytochemical analysis revealed the presence of major phytocompounds like terpenoids, alkaloids, glycosides, phenolics and tannins. Moreover, total flavonoid concentration equivalents to gallic acid was found in the range of 326 µgto 1481µg/g of plant extracts and that of total phenolic concentration equivalents to phenol was found in the range of 23.50 µg to 89.82µg/g of plant extracts. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

Introduction

Plants have enormous ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines) etc. It is now clear that, the medicinal values of these plants lie in the bioactive phytochemical constituents. They are also responsible to produce definite physiological effects.

A huge variety of medicinal plants and their purified components have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Gymnema sylvestre, Holarrhena antidysenterica, Vernonia anthelmintica, Enicostemma littorale, Momordica charantia, Swertia chirata, Azadirachta indica, Caesalpinia bonducella* and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarinlignans, catechins and isocatechins (Aquil *et al* 2006). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam *et al* 2004). Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins and so on (Edeoga *et al* 2005).

Oxygen is most essential element for all the living beings for their survival on this earth. Approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals during the process of oxygen utilization during normal metabolic and physiological processes. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10,000 oxidative hits per second (Mondal *et al* 2006). In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Lachman *et al* 1986).

It has been documented that most of the fruits, vegetables, natural plants contain a large variety of phytochemical and are the major source of antioxidant in the diet, which decreases the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexers of prooxidant metals, quenches of singlet oxygen etc. (Ebadi 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta *et al* 2004). Recently interest has increased considerably in finding natural occurring antioxidants for use in medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity (Kumaran *et al* 2007).

Materials and methods

Collection of plant materials

The samples were collected from the various places of Gujarat. It was ensured that the plant was healthy and uninfected. The samples were washed under running tap water to eliminate dust and other foreign particles and to clean the samples were then oven dried.

Preparation of leaf extracts

Ten grams of dried plant material was extracted with 100 ml of methanol kept on a rotary shaker for 24 hours. Thereafter, The extract was filtered using Whatmann filter paper No. 1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. These extracts were further subjected to the qualitative phytochemical analysis.

Phytochemical Analysis

The phytochemical tests were conducted using standard procedures to identify the constituents as described by Edeoga et al. (2005) and Harborne JB (1973). Tests for

the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, phlobatannins and phenols were carried out (Singleton *et al* 1974 and Marinova *et al* 2005).

Total Phenol Estimation

The total phenolic content of different extracts was measured using colorimetric Folin–Ciocalteu method (12). To 1 ml of methanolic extract, 5ml of distilled water was added. And further 250 µl of 1N folinciocalteau reagent was added. The mixture was covered and allowed it to stand for 3 min at 25°C. In this mixture, 1ml of saturated Na_2CO_3 and 1ml of distilled water were added. The mixture was left undisturbed for 1 hr at 25°C for color development and measured at 725 nm wavelength using a spectrophotometer. Standard graph was prepared by using different concentration of phenol crystals.

Total Flavonoid Estimation

Total flavonoid content was measured by aluminum chloride colorimetric assay (Koleva *et al* 2002). To 1 ml of methanolic extract 4 ml of distilled water was added. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Standard graph was prepared by using different concentration of gallic acid.

Determination of antioxidant activity by DPPH free radical scavenging assay

The free radical scavenging capacity of the crude extracts of the medicinal plants was determined using DPPH as described by Reena et al 2012. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts of plants were mixed with 95% methanol to prepare stock solution (1 mg/ml i.e. 1000 µg/ml concentration). The final volume was made up to 1 ml in all the tubes by the addition of ethanol. Then 3 ml of freshly prepared DPPH solution was added to all and were kept for 30 min incubation in the dark and absorbance was measured at 517 nm using UV- visible spectrophotometer. A stock solution of Ascorbic acid at the concentration 1000 µg/ml was prepared in distilled water. From this stock different concentrations ranging from 100-1000µl was prepared and used as the reference standard. The solution containing only methanol and DPPH served as the blank.(lee *et al* 2003, mathiesen *et al* 1995 and Kumar *et al* 2005).

% scavenging of the DPPH free radical was measured using the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of Control] (×) 100

Result and Discussion

Phytochemical screening of the extracts

The screening of plants for medicinal values has been carried out by number of workers with the help of preliminary phytochemical analysis. Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. Species of *Codiaeum variegatum* have been phytochemically investigated by Odukoya *et al* 2006.

The present study carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The phytochemical characters of all the plants investigated are summarized in the Table 1. The methanolic extracts of *Gymnema sylvestre* were found to contain saponin, terpenoids, flavonoids, phenol, cardiac glycosides and alkaloids. The methanol extracts of *Holarrhena antidysenterica* exhibit only tannins, phlobatannins, terpenoids, flavonoids, phenol, cardiac glycosides and alkaloids. The methanol extracts of *Vernonia anthelmintica* exhibit only tannins, phlobatannins, terpenoids, flavonoids, phenol, saponins and alkaloids. The methanol extracts of *Enicostemma littorale* exhibit only phlobatannins, terpenoids, flavonoids, phenol, cardiac glycosides, saponins and alkaloids. The methanol extracts of *Momordica charantia* exhibit only tannins, phlobatannins, terpenoids, flavonoids, phenol, cardiac glycosides and alkaloids. The methanol extracts of *Swertia chirata* exhibit only tannins, terpenoids, flavonoids, phenol, cardiac glycosides and alkaloids. The methanol extracts of *Azadirachta indica*exhibit only phlobatannins, tannins, terpenoids, flavonoids, phenol, cardiac glycosides.

Overall Studies of the phytochemical screening and qualitative estimation reveals that the parts of plant used were rich in phenol, flavonoids, terpenoids, and little cardiac glycosides. The plants studied here can be used as a potential source of useful drugs to cure various infectious diseases and was reported to cure infectious mastitis in bovine animals. (Reena et al 2013).

Total flavonoid contents of leaf extracts

The flavonoid content of the plant extracts was expressed in terms of gallic acid equivalent. The methanolic extracts of *Azadirachta indica* showed significant flavonoid content followed by *Swertia chirata and Holarrhena antidysenterica respectively*. However, least amount of flavonoid content was observed in *M. charantia* (Figure 1)

Total Phenolic contents of leaf extracts

The phenolic content of the plant extracts was expressed in terms of phenol equivalent. The methanolic extracts of *Azadirachta indica* showed significant phenol content followed by *Swertia chirata and Holarrhena antidysenterica respectively*. However, least amount of flavonoid content was observed in *M. charantia* (Figure 2). There is a positive correlation between phenolic content and free-radical scavenging activity (Oki *et al* 2002. The antioxidant potential of *Acacia nilotica* has been reported (Singh *et al* 2009). The high phenolic content of *A. nilotica* shows the linear correlation between phenolic content and antioxidant activity.

In-vitro Antioxidant activity DPPH scavenging activity

The free radical scavenging activity of plant extract was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH. It can react with it and thereby bleach the DPPH absorption (Reena *et al* 2012). When the plant extracts were tested for the DPPH free radical scavenging ability, the methanolic extract of *Azadirachta indica* and *M. charantia* at 1000µg/ml showed strong radical scavenging activity with percentage decrease of 96.50% and 95.25% whereas *G. sylvestre* showed relatively poor free radical scavenging activity of 78.70%.The order of scavenging activity was maximum in *A. indica* followed by *M. charantia,C.*

bonducella, Enicostemma littorale, Vernonia anthelmintica, Swertia chirata, Holarrhena antidysenterica, Gymnema sylvestre (Figure 3,4). The values are also comparable with commercial antioxidant L-ascorbic acid (90.0%). Maximum scavenging activity (57.10%) was observed at 100 µg/ml concentration and the IC50 value of gymnema extract (Rachh *et al* 2009). Holarrhenaantidysentrica, showed higher IC50 value as it had weaker DPPH scavenging activity. However, the chloroform and ethanol extracts showed less IC50 value comparable to the standard which had very less IC50 value (Shwetha *et al* 2011).

The phytochemical screening and qualitative estimation reveals that the medicinal plants used here were rich in phenol, terpenoids and little flavonoids, with antioxidant properties. Thus from these biochemical investigations, it is quite evident that all these species are very rich source of secondary metabolites.The plants studied here can be such as a potential source of useful drugs. In the present study, the metholic extract of *S. chirata*, showed higher IC50 value as it had weaker DPPH scavenging activity. The metholic extract of *H. antidysenterica*, showed least IC50 value as it had high DPPH scavenging activity. The methanolic extracts of various medicinal plants used which contain significant amount of flavonoid and phenolic compounds compared to the standards exhibited the significant differences in the antioxidant activity. This plant parts that we have studied here can be used to cure various infectious diseases of animals and humans.

Table.1 Results for photochemical screening

	Tannins	**Phlobatannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Cardiac glycosides**	**Alkaloids**	**Phenols**
S. chirata	+	-	-	+	+	+	+	+
V. anthelmintica	+	+	+	+	+	-	+	+
E. littorale	+	-	+	+	+	+	-	+
M. charantia	+	+	-	+	+	+	+	+
H. antidysenterica	+	+	-	+	+	+	+	+
C. bonducella	+	+	+	+	+	+	+	+
G. sylvestre	-	-	+	+	+	+	+	+
A. indica	+	+	-	+	+	+	-	+

(+) Present , (-) Absent

Fig.1 Total flavonoid contents among the medicinal plants

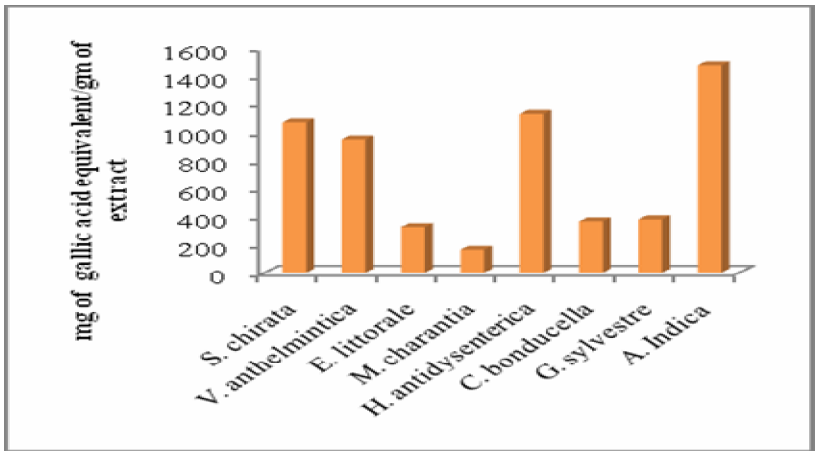


Fig.2 Total phenolic contents among the medicinal plants

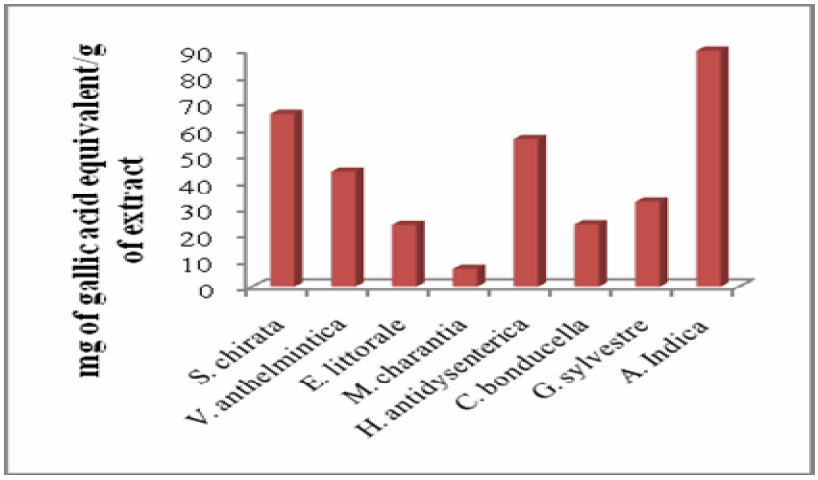
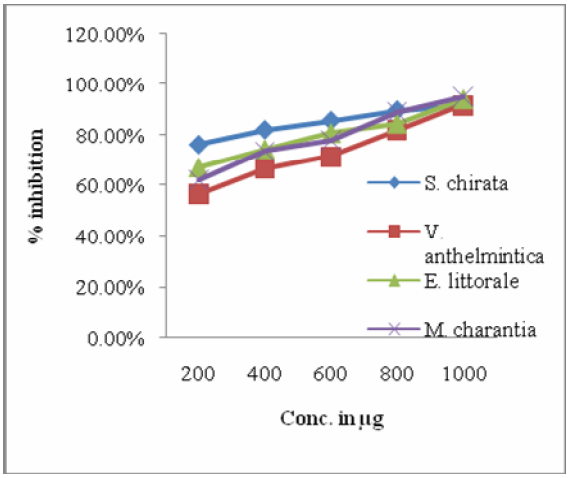
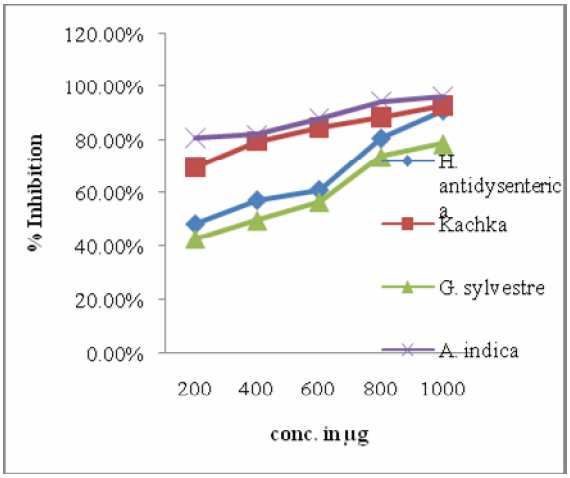


Fig.3 and **4** DPPH radical scavenging activity by different plant extract





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