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RESEARCH ARTICLE

Antioxidant Potential of Different Medicinal Plants

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ABSTRACT

Medicinal plants are the resource of new drug. Most of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Medicinal plants are the important sources for pharmaceutical manufacturing. In developing countries, herbal medicines are considered to be readily available, accessible, affordable, culturally acceptable and sustainable than Western medicines. In developed countries, the popularity of herbal medicines is continuing to grow, particularly for the treatment of certain disease. Medicinal plants have always been considered as a source of healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is natural. Researchers are increasingly turning their attention to natural products and looking for new, lead to develop better drugs. In the present study, the four different medicinal plants Zingiber officinalis, Terminalia arjuna, Punica granatum, Rauvolfia serpentine exerted antioxidative effects. The results suggest that aqueous extract of Terminalia arjuna possesses the potent antioxidant property when compared to other plant extracts. In turn, it has therapeutic potential for the prevention of coronary artery and renal diseases.

KEYWORDS

Medicinal plants, Reducing power assay, Nitrite, Lipid peroxide, DPPH

INTRODUCTION

India has a rich culture of medicinal herbs and spices, includes more than 2000 species and as a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines and only very few are studied chemically and pharmacologically for their potential medicinal value[1,2]. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care and attributes both biological and medicinal activities with lesser costs[3,4].

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Even with the advent of modern or allopathic medicine, Balick and Cox (1996)[5] noted that many important modern drugs are derived from plants. Traditional use of medicine is recognising potential future medicines. Researchers have identified number of compounds used in medicine were derived from ethno medical plant sources[6]. Medicinal plants are the source of many potent and powerful drugs[7,8].

In living systems, oxidation is the part of normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid) and free radicals (hydroxyl radical (OH) and superoxide anion) are generated[9-11]. Rapid production of free radicals causes alteration in the structure and function of cell constituents and

membranes, results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging[12-14]. These disorders can be overcome by the presence of antioxidants or the free radical scavenging molecules in the body.

There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites[15-18]. By taking foods rich in antioxidant compounds, lowers the risk of chronic health problems associated with the above disease conditions thereby maintaining the healthy body[14,19,20].

Zingiber officinale *i*s an important therapeutic herb in both the Chinese and Japanese systems of medicine, with fresh ginger being used for its warming properties and as a remedy for coughs and nausea while the dried product is indicated for ailments of the digestive system[21]. In more recent times, Z. officinale has undergone significant research as to its efficacy as a drug, particularly relating to its properties as an antiemetic, with studies showing that ginger is effective in reducing seasickness, vomiting in pregnancy and post-operative nausea and vomiting. Other studies have shown powdered ginger or ginger extract to be effective in reducing or relieving pain in patients with musculoskeletal disorders[22]. In aromatherapy, the essential oil of ginger is used for muscle and joint pain, sprains, colds, nausea, diarrhoea, alcoholism and helping the healing of broken bones[23].

Punica Granatum belongs to family Lythraceae, is a deciduous tree distributed throughout the world. Punica granatum generally called as Pomegranate. Punica granatum fruit is approximately 2.5-5 wide. It has red, leathery rind. Each seed is encased in pulp and sectioned of by walls[24]. It is considered as a pharmacy unto itself in ayurvedic medicine and is used as an antiparasitic agent, a blood tonic, and to heal ulcers. Its constituents include ellagic acid, ellagitannins, punicic acid, flavanoids, anthocyanidins, anthocyanin, estrogenic flavanols and flavones. Ellagic acid exhibits powerful anticarcinogenic and antioxidant properties[25]. It inhibits cyclooxygenase and lipooxygenase enzymes *in vitro*. Cyclooxygenase, a key enzyme in the conversion of arachidonic acid to prostaglandins, an important inflammatory mediator[26]. Punica granatum is shown to be antiatherogenic property by restoring the balance between the prooxidants and antioxidants.

Rauvolfia serpentina is an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. The plant belongs to the family Apocynaceae and occurs in habitats of tropical and subtropical regions[27]. The plant is commonly known as Sarpagandha, used in India as a part of the Ayurvedic medical system for the treatment of various ailments[28]. The potential of R. serpentina as antifungal, antiinflammatory, antioxidant, antiproliferative, anticancerous, antidiuretic, antifibrillar, antiarrhythmic, anticholinergic, antidysentry, antidiarrhoeal antihypotensive, anticontractile, antidiuretic, sympathomimetic, and tranquillizing agent. Phytochemical compounds or secondary metabolites present in R. serpentina include alkaloids, phenols, tannins and flavonoids. The plant parts, root and rhizome have been used since centuries in Ayurvedic medicines for curing a large number of diseases such as high blood pressure, mental agitation, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity[29-34].

The plant which has shown most promising and distinct results among these is Terminalia arjuna Wight & Arn., popularly known as arjuna[35]. The bark stem powder of this tree has been mentioned to be useful for “hritshool” (angina) and other related cardiac ailments by the ancient physicians. Recently there has been renewed interest in this plant because of its multimode

cardioprotective activity. Effect of arjunolic acid derived from Terminalia arjuna (15 mg/kg body weight) on antiplatelet activity, electrocardiographic changes, serum marker enzymes, antioxidant status, lipid peroxide and myeloperoxidase (MPO) were measured and compared with the acetyl salicylic acid (ASA) in rats subjected to isoproterenol challenge.

The drug was given intraperitoneally before and after isoproterenol administration. Arjunolic acid treatment prevented the decrease in the levels of SOD, CAT, glutathione peroxidase, ceruloplasmin, α-tocopherol, reduced glutathione (GSH), ascorbic acid, lipid peroxide and MPO. Cardioprotection conferred by arjunolic acid could possibly be due to the protective effect against the damage caused by myocardial necrosis[36]. Increase in serum CPK, SGOT, SGPT and following myocardial necrosis were significantly reversed by abana. The drug also showed 90% protection against reduction in glycogen levels in ischemic rats. The beneficial effect of abana was further evident by reduction in mitochondrial enzymes such as α-kG and succinate dehydrogenase (SDH) by 44% and 48%, respectively[37].

MATERIAL AND METHODS

Plant Material

The Medicinal plants were obtained from local ayurvedic market and were authenticated by the Botany department of BWC.

Extraction

The Medicinal plants were then powdered, dissolved in water and filtered. Aqueous extract (10%) was prepared according to the traditional system of medicine and about 100mg / ml filtrate is taken for the analysis.

Reducing power assay, nitrite, lipid peroxide and DPPH assay were performed in four different plants namely Zingiber officinalis, Punica granatum, Rauvolfia serpentine, and Terminalia arjuna. Phytochemical analysis of TA Bark as an *in vitro* study. 100mg / ml of the above said four different plant powder extracts were taken for the analysis.

Phytochemical Screening

Phytochemical screening of different medicinal plants was performed to test the presence of phenolic compounds, tannins, glycosides, saponins, alkaloids and flavanoids in the different plants using appropriate test[22].

Reducing Power Assay

The reducing power was determined by Oyaizu method (1986). Different concentrations of all the plant extracts (Zingiber officinalis, Punica granatum, Rauvolfia serpentina, Terminalia arjuna) (50-250 µg) in 1 ml of distilled water was mixed with phosphate buffer 92.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [K_3 $Fe(CN)_6$] (2.5 ml, 1%).

The mixture was incubated at 50^0 for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $Fecl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxyl toluene was used as a standard.

Nitric Oxide (No) Determination

Estimation of nitric oxide in terms of nitrite was based on the original method of Griess (1879) modified by Fiddler (1977) using the method of Isao et al., (1996). 50-250 µg of all the plant extracts was treated with 0.1ml of SSA mixed well and vortexed for every 5 minutes, for the total period of 30 minutes at room temperature.

0.5 ml of aliquot was taken with 1 ml of Griess reagent and allowed to stand for 20 minutes at room temperature, protected from light. The colour intensity developed was read at 420nm. Standards were also treated similarly. The results were expressed as nmoles/dl.

Estimation of Lipid Peroxide

Lipid peroxides were estimated by the method of Hunter et al., (1963).

The assay mixture contained 0.5 ml of homogenate, 9 ml of 0.15 ml of different concentrations of all the plant extracts. Lipid peroxidation was initiated by adding 100 ml of 1mm $FeCl_2$.

The reaction was stopped by adding 2 ml of ice-cold 0.25N HCL containing 15% TCA and 0.38% TBA as well as 0.2 ml of 0.05% butylated hydroxyl toluene. The reaction mixture was heated for 60 minutes at 80^0C, cooled to room temperature and centrifuged at 5000rpm for 15 minutes.

Optical density of the supernatant from each tube was measured at 532nm against a blank, which contained all reagents except liver homogenate and plant extract. Identical experiments were performed to determine the normal (without drug and ferric chloride) and induced (without drug) lipid peroxidation.

The percentage of antilipid peroxidation effect (% ALP) was calculated by following formula.

%ALP = Ferric chloride (OD) –Sample (OD) / Ferric chloride (OD) – Normal (OD) x 100.

Phytochemical Evaluation

Phytochemical examinations were carried out for the bark extracts as per the standard methods (Brain & Turner 1975, Evans 1996).

DPPH Free Radical Scavenging Activity

The free radical scavenging activity was followed by the DPPH method. 1ml of DPPH solution [0.1mmol in 95% ethanol (v/v)] was incubated with different concentrations of the extract.

The reaction mixture was shaken and incubated for 20min at room temperature and the absorbance was read at 517nm against a blank.

The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following equation.

DPPH Scavenged (%) = A control – A test / A control X 100

Statistical Analysis

All the values are represented as mean ± S.D (n=6). The statistical differences among different groups were analyzed by student's t-test. P-values of 0.05 or less were considered significant.

RESULTS

Table 1 shows the phytochemical analysis of the four different medicinal plants (Rauvolfia serpentina, Punica granatum, Terminalia arjuna, Zingiber officinalis) were carried out and of the four plants tested, TA demonstrated to possesses highest antioxidant activity compared to the standard ascorbic acid.

Table 2 shows the level of reducing power assay, nitrite lowering activity and lipid lowering activity in Zingiber officinalis, Punica granatum, Terminalia arjuna, and Rauvolfia serpentine. There is a significant increase in the level of reducing power assay, Nitrite, Lipid peroxide in Terminalia arjuna, when compared with Zingiber officinalis, Punica granatum, and Rauvolfia serpentine. There is no significant changes in the level of reducing power assay, Nitrite, and Lipid peroxide in Terminalia arjuna (100mg), when compared with control (ascorbic acid 50mg).

Table 3 shows phytochemical investigation of petroleum ether, methanol, chloroform, diethyl ether and aqueous extract of Terminalia arjuna revealed differences in their phytoconstituents. The results showed that alkaloids were highly present in non-polar solvents, tannins, saponins, triterpenoids, flavanoids were highly present in polar solvents. Moderate amount of diterpenoids and phlobotannins were present in polar and non-polar solvents.

Table 4 shows the DPPH scavenging activity was found to be in the range of (0.95-78.8) IP for Terminalia arjuna. It was observed that methanol extract showed highest scavenging (78.8 IP) & Petroleum ether showed the least activity (0.95 IP). From IP values, it was observed that antioxidant properties increases with increase in polarity of the solvent used for extraction.

Table 1: Detection of phytochemicals in the aqueous extract of various plant extracts

Name of the constituents	**Punica granatum**	**Rauvolfia serpentina**	**Zingiber officinalis**	**Terminalia arjuna**
Tannins	+	+	+	+
Saponins	-	+	-	+
Flavanoids	+	-	+	+
Anthocyanin	+	-	-	-
Alkaloids	+	+	-	-
Triterpenoid	-	-	-	+

(+) indicates present, (-) indicates absent

Table 2: The comparison of reducing power, Nitrite, and Lipid peroxide lowering activity

Plants	**Reducing power assay**	**Nitrite lowering activity**	**Lipid peroxide lowering activity**
Control (vit.C-50mg)	0.286 ± 0.10	0.115 ± 0.11	0.210 ± 0.09
Zingiber officinalis	0.238 ± 0.12	0.083 ± 0.07	0.092 ± 0.04
Punica granatum	0.246 ± 0.09	0.112 ± 0.13	0.044 ± 0.11
Terminalia arjuna	0.325 ± 0.11***	0.123 ± 0.05***	0.217 ± 0.07***
Rauvolfia serpentina	0.167 ± 0.03	0.07 ± 0.02	0.188 ± 0.31

Values are expressed as mean ± S.D.

Values are compared with control and TA

*** P > 0.001%.

Table 3: Phytochemical analysis of the TA bark extracts

Components	Petroleum ether	Chloroform	Methanol	Diethyl ether	Water
Carbohydrates	-	+	+	+	+
Proteins	-	-	-	-	-
Aminoacids	-	-	-	-	-
Lipids	+	+	+	+	-
Alkaloids	+++	+++	+	++	-
Flavonoids	-	+	+++	-	+
Glycosides	+++	+++	++	++	++
Phenols	-	+	++	+	++
Saponins	-	-	++	+	+++
Sterols	++	+	-	+	-
Terpenoids	++	+	++	++	++
Diterpenoids	-	+	-	+	-
Triterpenoids	-	-	++	+++	-
Tannins	-	+	-	+++	+++
Phlobotannins	-	-	+	-	+
Anthraquinones	-	-	-	-	-
Fixed oils & fats	-	-	-	-	-
Resins	-	-	-	-	-

Table 4: *In vitro* antioxidant activity of TA bark extracts

Extracts	DPPH free radical scavenging activity
Petroleum ether	0.95 ± 0.03
Chloroform	1.06 ± 0.06
Methanol	78.8 ± 1.08
Diethyl ether	2.11 ± 0.02
Water	10.3 ± 0.86

DPPH Values are expressed as mean ± S.D

Values are compared with control & Terminalia arjuna

DISCUSSION

Many plant derived substances collectively termed phytonutrients or phytochemicals are becoming increasingly known for the antioxidant activity. Phenolic compounds such as flavanoids are ubiquitous, with in the plant kingdom; approximately 3000 falvanoid substances have been described. In plants, flavanoids serve as protectors against a wide variety of environmental stresses while in humans, flavanoids appear to function as biological response modifiers. Flavanoids have been demonstrated to have anti-inflammatory, antiallergic, antiviral and anticarcinogenic activity[38].

The broad therapeutic effects of flavanoids can be largely attributed to their antioxidant property. In addition to an antioxidant effect, flavanoids may exert protection against heart disease through the inhibition of cyclo oxygenase & lipo oxygenase activities in platelets and macrophages[39].

In the present study, the phytochemical analysis of the four different plant extracts (Zingiber officinalis, Punica granatum, Terminalia arjuna, Rauvolfia serpentina) were carried out and their antioxidant activity were investigated in *in vitro* condition and of the four plants tested, TA demonstrated to possesses the highest antioxidant activity, when compared to the standard ascorbic acid. TA was selected as the best among all, because of its beneficial effect.

The phytochemical screening of TA indicates the presence of phytosterols (b-sitosterol), flavanoids (arjunone, arjunolone, and luteolin) in different bark extracts. All the extracts were negative for amino acid and resins. Only the diethyl ether extract of bark showed the presence of triterpenoids that are effective as that of vitamin C dose in protecting the rat heart against CsA toxic insult. The saponin compounds may be responsible for inotropic effects of TA. The data obtained was consistent with Row et al., 1970[40] and Sharma et al., 1982[41], who had also mentioned the presence of tannins, flavanoids and triterpenoids in the bark.

The DPPH radical scavenging activity was found for different extracts of TA. The methanolic extract gave highest scavenging activity and petroleum ether gave the least. Hydrogen donating ability of the antioxidant molecule contributes to its free radical scavenging nature[42].

It has been reported that the flavanoids and OPC's (oligomeric proanthocyanidins) provide free radical scavenging and antioxidant activity of vascular strengthening. No significant untoward effects were reported during TA therapy, which allows as going in for a deeper research in the study of cellular mechanism helping in cardio and renoprotective effect.

CONCLUSION

The preliminary phytochemical analysis revealed the presence of various components such as tannins, saponins, triterpenoids and flavanoids in the aqueous extract of Terminalia arjuna. The beneficial effect of TA is due to its antioxidant property. This study indicates that TA possesses antioxidant, reducing power, nitrite, DPPH free radical scavenging activity and can be developed as the pharmaceutical drug in the near future.

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