EVALUATION OF ANTIOXIDANT ACTIVITY OF *GMELINA ARBOREA* EXTRACTS BY *IN VITRO* TECHNIQUES

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Summary

Plants are considered to be a promising source of medicine in the traditional health care system. Now days, there is a revival of interest with herbal-based medicine due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine. *Gmelina arborea* also known as gambhar has a huge potential in herbal medicine for its anti-oxidant, antiviral and many other pharmacological properties. In the present study the antioxidant activity of bark of *Gmelina arborea* was studied by various *In vitro* techniques. Phytochemical studies were performed on the extracts, Aqueous extract (AE**)** and Methanolic extract (ME). Assays performed were Free radical scavenging assay, Reducing power assay and Nitric oxide screening activity. The study revealed that the plant *Gmelina arborea* possesses antioxidant activity. The antioxidant activity of *Gmelina arborea* is mainly due to presence of phenolic compounds.

Keywords: Antioxidant activity, Free radicals, DPPH, *Gmelina arborea*

Introduction

Oxygen is of prime importance for the survival of all species on this earth. During the process of oxygen utilization in a normal physiological and metabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals[1]. In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders[2]. Free radicals are atoms that have at least one unpaired electron, thus making them unstable and highly reactive. Free radicals then roam throughout the body causing damage to cells by stealing stable electron partners from other cells, causing more free radicals, more instability, and more damage. This cell damage can impair the body's ability to fight illness[3]. A typical example of oxygen free radicals is $O^{\cdot}_2$ -. "Reactive oxygen species" is a collective name containing some non-free radical molecules that can also induce lipid oxidation. The examples of reactive oxygen but non-free radicals are hydrogen peroxide (H_2O_2), hypocholorous acid (HOCl), ozone (O_3), and singlet oxygen ($^1O_2$). The reactive oxygen species belonging to free radicals include superoxide negative ion radical ($O^{\cdot}_2$ -), hydroxyl free radical ($OH^{\cdot}$, a very powerful oxidization agent), peroxyl ($ROO^{\cdot}$), and alkoxyl ($RO^{\cdot}$). Human cells have an array of protecting mechanisms to prevent the production of free radicals and oxidative damage.

These mechanisms include both enzymic and non enzymic antioxidants such as superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherols. The protective roles of these enzymes may be disrupted as a result of various pathological processes and thereby causes damage to the cells[4].

Antioxidants are free radical scavengers which protect the human body against free radicals[2]. 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[1]. Most living species have a number of defense and protective mechanisms against the oxidative stress and toxic effects of normal oxygen metabolism. However, those protective systems gradually decrease with resulting in disturbances to the normal redox equilibrium established in healthy system. Thus, the antioxidants that regulate the various oxidative reactions are found naturally in tissues and are evaluated as a potential class of longevity determinant[5]. Recently extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternatives medicines for the treatment of many infectious diseases and also in preservation of food from the toxic effects of oxidants. In modern days the antioxidants and antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy. Because of the possible toxication of synthetic antioxidants like-Butylated hydroyl anisole (BHA) and Butylated hydroxyl toluene (BHT), an increased attention has been directed towards natural antioxidants[6]. It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease 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[1]. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals . Plants based natural constituents like phenolics can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc. these phenolics act as antioxidants. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct[2].

An unarmed (without branches) tree has about 60 ft. height. It is found scattered in deciduous forests throughout the greater part of India and the Andamans upto an altitude of 5000 ft. It is also planted in gardens and avenues[7].

The root, fruit, bark and leaves of the plant are being used in medicine[7,8]. The root is stomachic, laxative, anthelmintic; improves the apetite; useful in hallucinations, thirst, piles, abdominal pains, burning sensations, fevers, *tridosha*[9]. Root is an ingredient of the Dasamulas of the vaidyas. It is used in the form of infusion or decoction in fever, in indigestion, anasarca. With liquorice, sugar and honey added it is given as galactogogue[10]. The root bark improves thirst and relieves abdominal pain [11]. Pulverised root is applied locally for gout[7]. The flowers are sweet, cooling, bitter, acrid; astringent; useful in leprosy and blood diseases[9]. The fruit is acrid, sour, bitter, cooling, diuretic, aphrodisiac, alterative, astringent to the bowels. It promotes growth of hairs, useful in *vata*, thirst, anaemia, leprosy, ulcers, consumption, vaginal discharges[9]. The plant is used in treatment of diabetes[12,13,14], in snake-bite and scorpion sting [10,15]. In snake-bite a decoction of the roots and bark (1 in 16) is given internally[9].

Materials and Methods

Chemicals: L-Ascorbic acid, 1,1-diphenyl -2- picryl hydrazyl were purchased from sigma-Aldrich, USA. Potassium ferricyanide, Trichloro acetic acid, Ferric chloride, Sodium Nitroprusside, Glacial acetic acid were purchased from Qualigens, India. Sulphanilic acid, 1-Napthyl amine were purched from Sisco research lab., India

Plant Materials and Preparation of Extracts: The Bark of the plant was collected from Jawhar, Dist. Thane. India and were authenticated by scientists of Botanical survey of India, Pune. Bark was shade dried and then powdered. Two extracts were prepared, Aqueous extract was prepared by cold maceration wit water and was concentrated to dryness. Methanolic extract was prepared by soxhlet extraction followed by concentration to dryness by rota - evaporator under reduced pressure.

Free radical scavenging assay: DPPH solution (0.2 mM, 1ml) was added to 3ml of extract solution of different concentrations (30–60 µg/ml for ME and 90-150 µg/ml for AE). The mixture was vigorously shaken and allowed to stand at room temperature for 10 min. The absorbance was measured at 517 nm in a spectrophotometer[16]. Ascorbic acid was taken as control and procedure was repeated. Percent radical scavenging activity was calculated by using formula.

$$\% \text{ Radical Scavenging activity} = \frac{Abs_{Con} - Abs_{Sam}}{Abs_{Con}} \times 100$$

Reducing power assay[16]: Various concentrations of the extracts (30 to 60µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). Absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (10 to18 µg/ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. % increase in activity was calculated by using formula,

$$\% \text{ increase in Reducing Power} = [(A_{test}/A_{blank}) - 1] * 100$$

Where,

A_{test} = absorbance of test solution

A_{blank} = absorbance of blank

Nitric oxide Screening activity[17]: The reaction mixture (3 ml), containing sodium nitroprussude (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and sample extract (30–60 µg/ml for ME and 90-150 µg/ml for AE) or standard solution (0.5 ml) was incubated at 25ºC for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and 1ml of sulphanilic acid reagent (0.33%) was added & mixed. Above mixture was allowed to stand for 5 mins for completion of diazotization.

1ml of naphthylamine (1%) was added to the mixture and mixed and allowed to stand for 30 min. Absorbances of these solutions were measured at 540 nm against the corresponding blank solutions. Percent radical scavenging activity was calculated by using formula,

$$\% \text{ Radical Scavenging Activity} = \frac{\text{Abs}_{\text{Con}} - \text{Abs}_{\text{Sam}}}{\text{Abs}_{\text{Con}}} \times 100$$

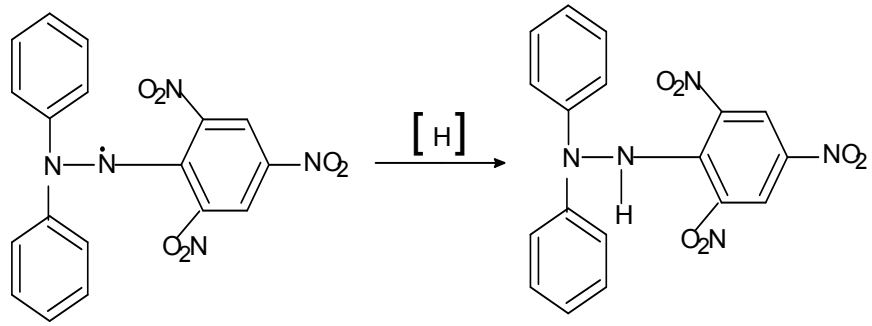
Where,

Abs_{Con} is absorbance of Control,

Abs_{Sam} is absorbance of Sample solution.

Results and discussion

Free radical Scavenging assay[18,19]**:** The DPPH antioxidant assay is based on the principle that 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, is able to decolourise in the presence of free radical scavengers (antioxidants). The odd electron in the DPPH radical is responsible for the absorbance at 517 nm, and also for visible deep purple colour. When DPPH accepts an electron donated by a free radical scavenger, the DPPH is decolorized, and the extent of decolourisation can be quantitatively measured from the changes in absorbance.



It was found that the radical- scavenging activities of both extracts increased with increase in concentrations of extracts. It showed a significant activity. IC_{50} for DPPH radical-scavenging activity was 35µg/ml for ME and 100µg/ml for AE. The IC_{50} values for Ascorbic acid were 16µg/ml. The significant activity of *Gmelina arborea* suggests that hydrogen donation maybe a possible mechanism for antioxidant activity of this plant.

Reducing power assay[20]**:** Substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}). Potassium ferrocyanide then reacts with ferric chloride to form ferric ferrous complex. This complex has an absorption maximum at 700 nm. It was found that the reducing powers of both the extracts also increased with the increase of their concentrations. Extracts showed a substantial activity. Figure 1 shows an increase in reducing power activity with respect to concentrations.

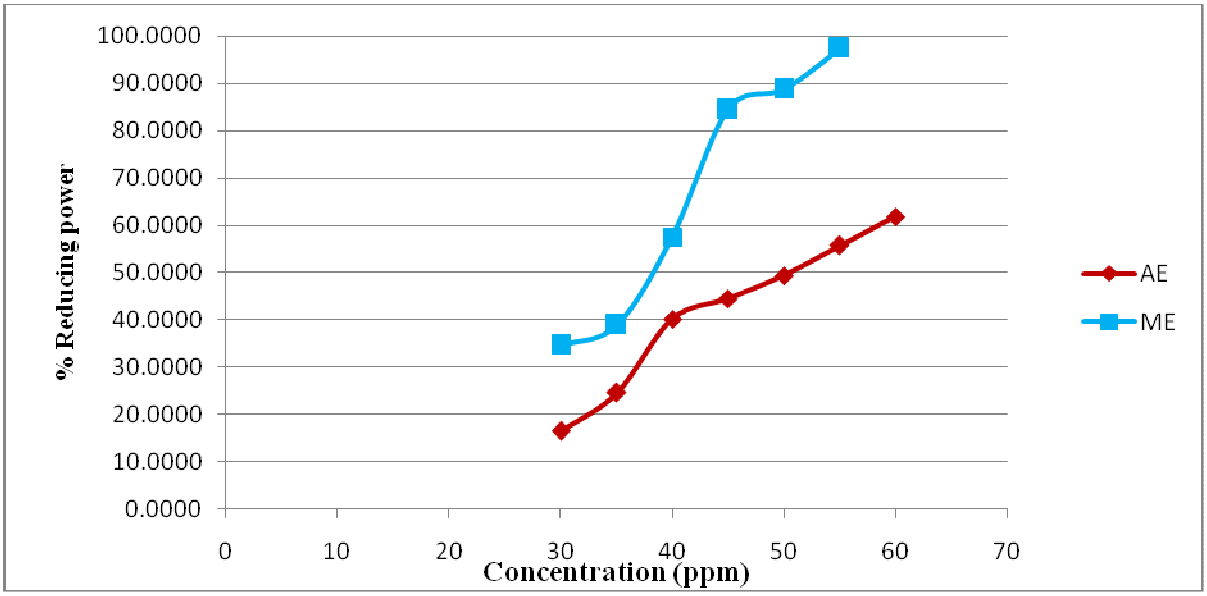


Figure 1: Reducing power of *Gmelina arborea* of AE and ME

The IC_{50} value for ME was found to be 40µg/ml and for AE was 55µg/ml.

Nitric oxide Screening activity[16]**:** Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, reacts with molecular oxygen to form nitrite ions. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide, which can be estimated by the use of Griess Illosvoy reaction. Sulfanilamide is quantitatively converted to a diazonium salt by reacting with nitrite in acidic conditions. This diazonium salt coupled with 1-napthylamine; forming an azo dye that can be measured quantitatively at 540nm. The ME and AE extracts of *Gmelina arborea* showed moderately good nitric oxide-scavenging activity1. The percentage of inhibition increased with increasing concentration of the extract. IC50 was calculated as 60µg/ml and 140µg/ml. However, activity of ascorbic acid was very more pronounced than that of our extract (IC50= 18 µg/ ml).

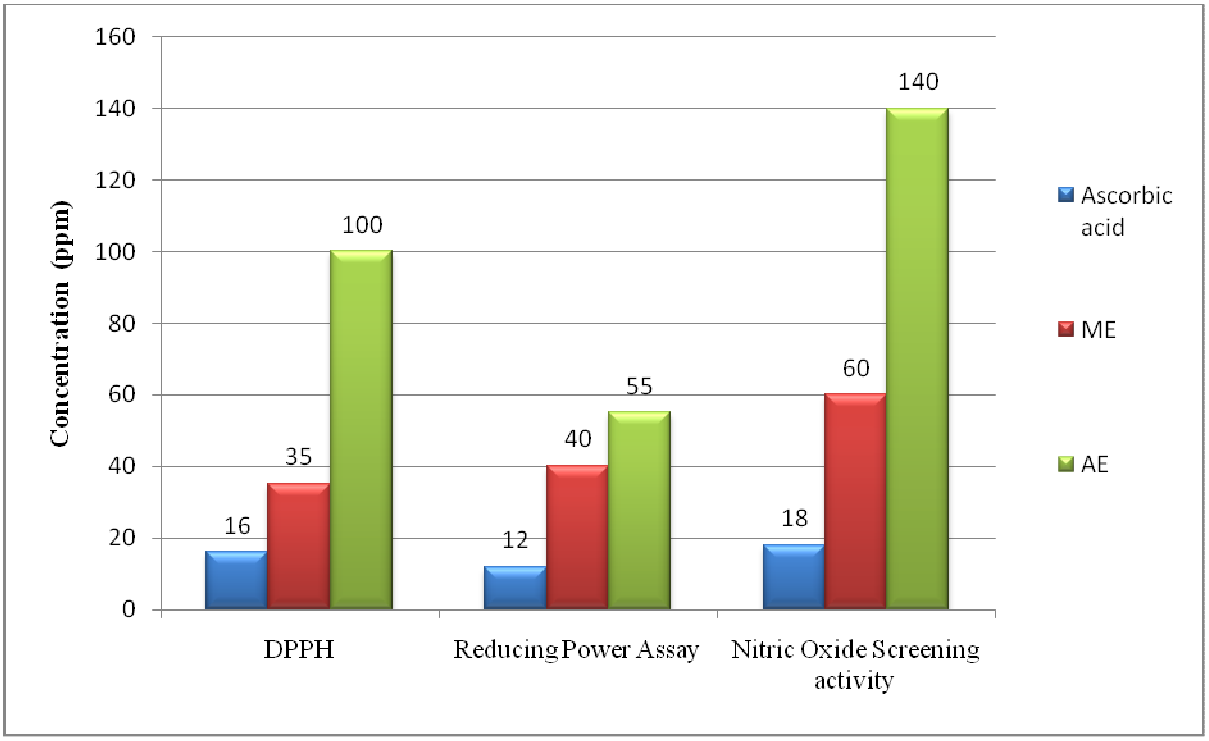


Figure 2: IC_{50} values of standard (ascorbic acid) and both the extracts (ME & AE) for DPPH, Reducing power assay and Nitric oxide screening activity.

The above figure (Figure 2) summarizes the IC_{50} values of the extracts used as well as that of standard.

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