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EVALUATION OF ANTIOXIDANT POTENTIAL OF *HOPEA PONGA* BARK EXTRACTS.

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ABSTRACT

Hopea ponga, an ethanomedicinal plant which is known to its wide variety of medicinal value in the folk medicine. In this paper, we demonstrated the antioxidant potential of *Hopea ponga* bark extracts by using different solvent system. Crude extracts of *Hopea ponga* were used perform an *invitro* antioxidant assays such as DPPH, Ferric ion reducing capacity, phosphomolybdenum and H_2O_2 activity. Among these extracts, acetone extracts posses a promising antioxidant activity followed by water>methanol>ethyl acetate> alcohol against standard, L-ascorbic acid. At 100µg concentration acetone shows 96.03% & 96.45% of inhibition in DPPH radical scavenging activity and H_2O_2 activity respectively. Further study requires elucidating / determining the active principles present in the plant.

KEYWORDS: *Hopea ponga*, free radicals, DPPH, phytochemicals, etc.

INTRODUCTION

Reactive oxygen species (ROS) are known chemical reactive molecules derived from the oxygen, which is generated during normal metabolic process in the cell system. The ROS includes hydroxyl radicals, hydrogen peroxide, nitric oxide radicals etc are readily react with various biomolecules such as proteins, carbohydrates, nucleic acids (DNA) and so on (Droge et al., 2002, Hippeli & Elstner et al., 1999). Increases in the concentration of free radicals leads to oxidative damage & finally affects cellular metabolism leads to chronic disease such

as cancer, arthritis, inflammation & cardiovascular disorders (Maxwell et al., 1995, Black et al., 1995). Consequently, both enzymatic and non enzymatic antioxidants that neutralize the free radicals effect on the cell and protecting the human body from such diseases. There many synthetic antioxidants are restricted, due to its toxic and carcinogenic effect in the cellular system. Plants are fulfilling these problems due to its easy availability and nontoxic in nature. Presence of phytochemicals such as polyphenols, flavonoids, proteins, tannins, saponins exhibits a potent antioxidant activity (Hill AF et al., 1952). There are many reports has been made that antioxidant potential of medicinal plant that allow them to act as a reducing agent, hydrogen donator and free radical quencher (Shahidi F et al., 1992).

Hopea ponga, belongs to Dipterocarpaceae family & endemic tree in IUCN red list of threatened species. It is commonly found in tropical evergreen forest including Malaysia & Western Ghats of India (Shiddamallayya N et al., 2008). The different parts of the plant have been used for the treatment of piles and snake bite in the folk medicine. The bark of the tree is a good tanning material and astringent (sukesh et al., 2011) The antibacterial and antioxidant activity of the seed wings are reported (Rose et al., 2013) There are no reports has been made on the biological properties of the *Hopea ponga* bark extract. Here we first time demonstrated the antioxidant property of the *Hopea ponga* bark extract.

MATERIALS AND METHODS

Chemicals

DPPH Sigma (St. Louis, USA). Potassium ferrocyanide, L-Ascorbic acid from Hi-media. Phospho-molybdenum (SRL), Hydrogen peroxide, ferrous sulphate, ferric chloride was procured from Merk.

Experimental Design

Materials and Methods

Plant bark was collected from Madikeri District, Karnataka. The collected bark was cut into small pieces (2-3cm) and dried under shade. Dried material was finely powdered by using electric blender and collected in an air tight container until for the next use. The powdered (1 gm) of was extracted 100ml of different solvent systems like water, alcohol, methanol, ethyl acetate & acetone for overnight in orbital shaker at room temperature. The extracts were filtered through No.1 whattmann filter paper. The filtrate was concentrated by using flash evaporator and collected, stored at 4°C. The samples were prepared by dissolving 1mg of samples in 1ml of 10% DMSO at concentration 1µg/µl and used for assays.

Phytochemical Components

Phytochemical analyses were carried out according to the methods described by Trease and Evans et al., 1989 of the crude powder of bark for the identification of phytochemicals like tannins, alkaloid, steroids, saponins and flavonoids.

Antioxidant assays

DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts was determined according to the method reported by Gyamfi et al., 1999. Different concentration of plant samples (20µg-100µg) was taken and mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-Hcl buffer (pH 7.4). Methanol (50 µl) only was used as the experimental control. After 30 min of incubation at room temperature, the percentage of inhibition was measured by reading the absorbance at 517nm. L-Ascorbic acid were used as controls. The percent inhibition was calculated from the following equation:

$$\% \, of \, inhibition = C - T/C$$

Where **C** is Control & **T** is Test.

Phosphomolybdenum activity

The phosphomolybdenum activity of the different solvent extracts of Hopea ponga was carried out according to the method Prieto et al., 1999 with minor modifications. The reaction mixture contains standard L-ascorbic acid & different concentration of extracts (20µg-100µg) was mixed with 1ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and incubated in a water bath (95^0C for 90 min). The absorbance of the mixture was measured at 695 nm. The different concentration of L-ascorbic acid was used as positive control and experiment was performed as above mentioned protocol.

Ferric ion reducing activity *(Fe^{3+} to Fe^{2+})*

The ferric ion reducing capacity was determined according to the method of Wang et al., 2003 with minor modifications. The reaction mixture contains standard L-ascorbic acid & different concentration of extracts (20µg-100µg/mL) was mixed 100µl of potassium ferricyanide solution (4mM) was mixed with 200µl of 20mM phosphate buffer, pH 6.5, with or without test samples. The reaction mixture was incubated at 50°C for 20 min. 10% of 200µl of tri-chloro acetic acid was added to the reaction mixture and centrifuged at 5319g.

The resulting supernatant was taken and mixed with 100µl of ferric chloride solution (2mM) and final volume was made up to 1 ml with water and then incubated at room temperature for 10 min. The absorbance was recorded at 700nm by using spectrophotometer. Absorbance increases with an increase in ferric ion reducing capacity.

H_2O_2 activity

The ability of the test sample to scavenge hydrogen peroxide was determined according to the method given by Ruch et al.,1989. The reaction mixture contains 0.6mL of 2mM of hydrogen peroxide in phosphate buffer were incubated with different concentration of test sample (20-100 µg/ml) Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

$$\% \, of \, inhibition = C - T/C$$

Where **C** is Control & **T** is Test.

RESULTS AND DISCUSSION

Medicinal plants components which can either sacrificially scavenge free radicals to inhibit the radical chain reactions, considered as primary chain breaking antioxidants or preventive antioxidants (Karadag A et al., 2009). But there is no single **report** has been proven the mechanism of action of all **radicals** sources or all antioxidants in a complex system. (Prior RL et al., 2005). Due to various oxidative processes occur in cellular level, the antioxidant capacity of a sample has been achieved by various mechanisms like reductive and radical scavenging activity, prevention of chain initiation, decomposition of peroxides, etc. The phytochemicals compounds are known powerful chain breaking antioxidants (Shahidi F et al., 1992), due to the presence of hydroxyl groups and contribute directly to antioxidative action (Suresh Kumar et al., 2008). These phyto constituents are also effective hydrogen donors, which makes them good antioxidants. It suggests that phyto constituents have inhibitory effects on mutagenesis and carcinogenesis in humans.

Table 1: Phytoconstituents evaluation of *Hopea ponga* bark extracts.

Phytochemical constituents	Water	Ethanol	Methanol	Ethyl acetate	Acetone
Polyphenols	+	+	+	+	+
Steroids	-	+	+	+	+
Saponins	-	+	+	+	+
Sugars	+	+	+	+	+

Flavonoids	+	+	+	+	+
Proteins	+	-	+	-	+

The Phytochemical analysis of *Hopea ponga* bark extracts has been demonstrated in table1. This shows the bark was enriched with various phytochemicals. The presence of these constituents is responsible for exhibiting an antioxidant and other biological property.

The *invitro* antioxidant activity of the sequential extracts (polar to non-polar) of *Hopea ponga* bark extracts has been evaluated by using various assays such as DPPH, phosphomolybdenum assay, ferric ion reducing assay and hydrogen peroxide activity. Among these extracts, maximum antioxidant activity was observed in acetone extracts. The results indicate the choice of the solvent extracts for the future elucidation of the active principles involve in the scavenging activity. The presence of various phytochemicals is responsible for scavenging the free radicals.

DPPH radical scavenging activity

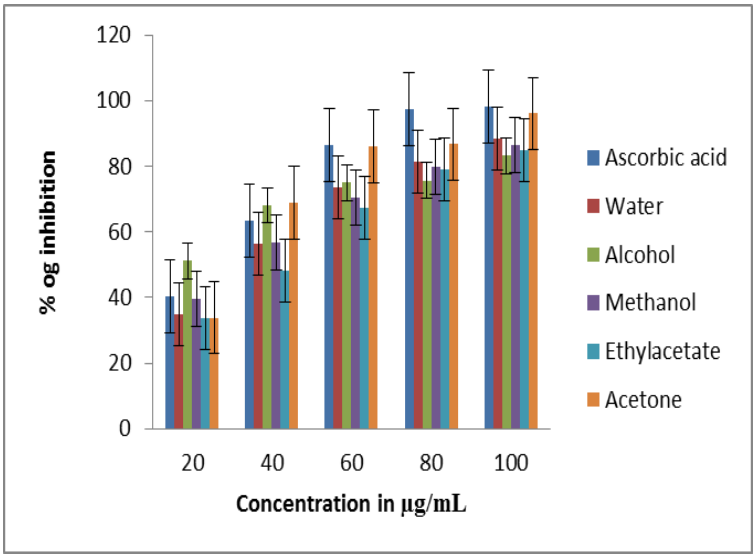

Fig 1: DPPH radical scavenging activity

The scavenging activity of different extracts of test sample has been shown in Fig1. The extracts were comparable with standard antioxidant L-ascorbic acid/ Vitamin –C in a dose dependent manner. At 100µg concentration acetone extract exhibit a maximum inhibitory activity up to 96.03% followed by water>methanol>ethyl acetate>alcohol. These extracts show slightly less inhibitory activity against standard antioxidant L-ascorbic acid (98.12%). The effect of donor & acceptor hydrogen species has been attributed by DPPH radical scavenging activity. The stable violet/ pink colour DPPH solution in methanol has been

undergo colourless solution due to the decreasing quantity of DPPH radicals in the environment. The discolouration of solution reflects the radical scavenging activity of the test sample (Guo et al., 2007).

Phosphomolybdenum activity

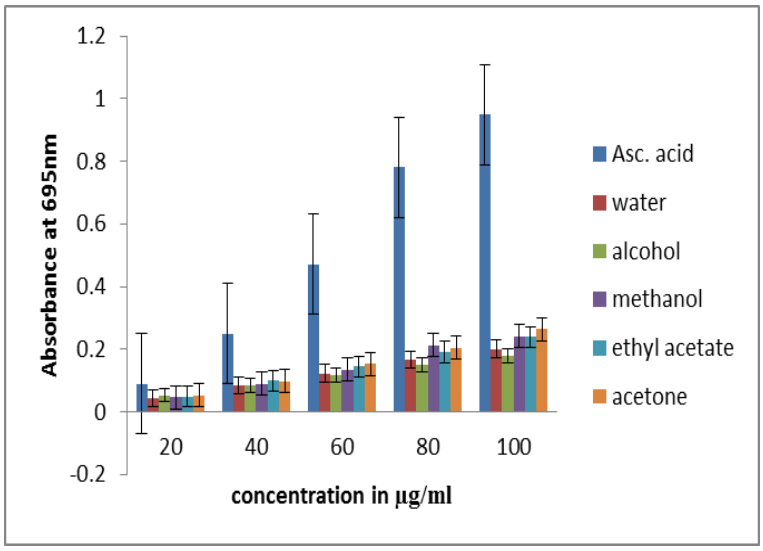

Fig 2: Phosphomolybdenum activity

The total antioxidant activity of the test sample has been determined by using phosphomolybdenum assay which has been depicted in Fig2. Mechanism involves the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/ Mo (V) complex at acidic pH. It also evaluates water soluble and fat soluble antioxidant present in the sample (total antioxidant activity: TAC). The acetone extract shows the high antioxidant capacity when compare to other extracts, but lower than the standard antioxidant L-ascorbic acid. This suggests the antioxidant potential of the extract, because antioxidant capacity of ascorbic acid has used as a reference with which plant extracts with potential antioxidants are compared (Aderogba M.A et al., 2005).

Ferric ion reducing assay

The reducing capacity of the test sample has been depicted in Fig3. It was suggested that the reducing & electron donating capacity of the bioactive compounds, is associated with antioxidant activity. The presence of such reductants causes the reduction of the ferric/Fe^{3+} complex to the ferrous Fe^{2+} form (Tuba A.K et al., 2008). The reducing power of the test sample increases with increases in concentration of test sample, which suggests that the electron donating capacity of the test samples in a dose dependent manner. Again acetone

extract shows the promising reducing ability compare to other extracts as well as standard, ascorbic acid.

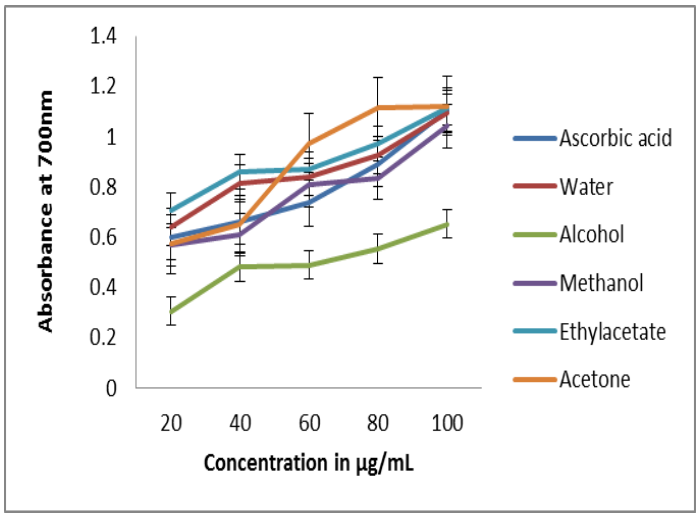

Fig 3: Ferric ion reducing assay

Hydrogen peroxide activity

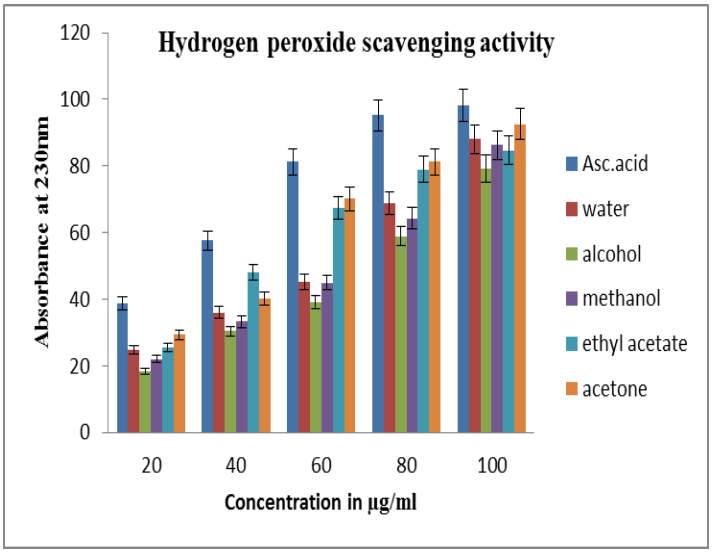

Fig 4: Hydrogen peroxide activity

The scavenging ability of the test sample against standard has been shown in Fig4. The extracts were comparable with standard antioxidant L-ascorbic acid/ Vitamin–C in a dose dependent manner. At 100µg concentration acetone extract exhibit a maximum inhibitory activity up to 92.56% followed by water>methanol>ethyl acetate>alcohol. These extracts show slightly less inhibitory activity against standard antioxidant L-ascorbic acid (98.21%). Hydroxyls radical (OH-) produced from hydrogen peroxide are toxic at sub cellular level

(Halliwell et al., 1981 & Halliwell et al., 1987). Thus removal of H_2O_2 is very important is very important for antioxidant defence in cellular system.

CONCLUSION

Different solvent extracts of *Hopea ponga* for antioxidant activity has been determined by using various invitro assays. Among them acetone extracts shows a promising activity. Due to the presence of wide variety of phytochemicals such as polyphenols, flavonoids, sugars, tannins, saponins, proteins, etc involves in the exhibiting antioxidant activity. The present investigation offers scientific evidence to the use of bark of *Hopea ponga* in the folkloric accounts.

Conflict of interest: The authors declare that they have no conflict of interest.

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