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FREE RADICAL SCAVENGING EFFECT AND PHYTOCHEMICAL ANALYSIS OF LEAVES EXTRACT OF PLECTRANTHUS AMBONICUS

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ABSTRACT

Indian medicinal plants are playing a vital role in the preparation of biomedicine. Most of the herbals are used in siddha medicine for the different pharmacological applications such as antibacterial, antifungal, antioxidant, controlling od diabetes, cardio protective, anticancer and anti-inflammatory activities. In this present investigation, we have used very important medicinal plant karpooravalli (Plectranthus ambonicus) for the biomedical applications like free radical scavenging activity. The free radical scavenging activity was conducted by non enzymatic methods, for that we have used the methodologies such as DPPH radical assay, Nitric oxide radical inhibition assay, Superoxide anion scavenging activity, Hydrogen peroxide-scavenging activity, Estimation of lipid peroxidation inhibition, ABTS radical scavenging activity, Hydroxyl radical scavenging assay and Reducing power analyzed. The results are showing good peaks may confirm the activities. Phytochemical screening also conducted for Alkaloids, tannins (Ferric chloride test), saponins, glycosides, flavonoids, protein, steroids, terpenoids, triterpenoids, sugars, and phenol. Finally, the In-vitro anticancer activity against Hep G2 Liver cell line culture analyzed using Assay for cytotoxic activity (MTT assay). The study proves the very good biomedical action of karpooravalli plant leaves extract.

KEYWORDS: *P. Ambonicus, DPPH, ABTS, Nitricoxide, Superoxide Hydrogen Peroxide, Alkaloids, Plectranthus Amboinicus, Antioxidant Activity, Free Radical Scavenging Activity, Phytochemical Analysis*

INTRODUCTION

- Description: Plectranthus amboinicus
- Systematic classification of Plectranthus amboinicus
- Kingdom: Plantae Division: Magnoliophytes Order: Lamiales
- Family: Lamiaceae
- Genus: Plectranthus
- Species: amboinicus (Lour.) Spreng.

Figure 1: Leaves of Plectranthus Amboinicus

P. amboinicus Lour known as Indian Borage belongs to the Lamiaceae family, and it is a commonly available medicinal plant. In Tamil it is called as omavalli or karpooravalli. It is a small herb, 30-90 cm in height with fleshy leaves and cultivated throughout India. It is a highly aromatic pubescent herb with distinctive smelling medicinal plant contains many phytochemicals such as carvacrol (monoterpenoid), caryophyllene (bicyclic sesquiterpene), patchoulene, andflavonoidslike quercetin, apigenin, luteolin, salvigenin, and genkwanin. It is used for the treatment of malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic, and convulsions. It is also known to be a very powerful painkiller, stimulates the flow of bile aiding digestion. The leaves are said to have a specific action on the bladder and to be very useful in urinary disease and vaginal discharges (Rao et al., 2006). Theyareuseful in cephalalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, and cholera especially in children, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccough, bronchitis, renal and vesical calculi, strangury, hepatopathy, malarial fever, antispasmodic and cathartic. Juice of leaves mixed with sugar acts as a powerful aromatic carminative, given in colic and dyspepsia. Crushed leaves are used as a local application to the head in headache and to relieve the pain and irritation caused by stings of centipedes (Gurgel et al., 2009, Jia-Ming et al., 2010, Kaliappan et al., 2008).

In this present investigation phytochemical screening is performed to detect the presence of a potential bioactive component in the aqueous extract and perform free radical scavenging activity using the aqueous extract ofP. amboinicus.

MATERIALS AND METHODS

Preparation of Leaves Extract

Plant Material

Leaves of *Plectranthus amboinicus* (Lour.) was collected locally in and around Vandavasi and confirmed and authenticated by Mr.Raja, Botanist Santhimalai research foundation.

Extract Preparation

Leaf of Plectranthus amboinicus (Lour.) was washed with water to remove soil particles and other impurities. A 20 gram of plant leaves were cut into small pieces and extracted by boiling with water for 30 min at 90 °C. Then the boiled

solution was filtered through Whatman No. 1 filter paper. Collect the filtrate and allowed to evaporate to obtain crude extract. For further experiment crude extract was diluted with water at the concentration of 1mg/1 ml and stored for further use.

Phytochemical Screening

The obtained aqueous extract was analyzed to preliminary phytochemical screening following the standard protocols.

Non-Enzymatic Antioxidant Assay (Free Radical Scavenging Assay)

The standard procedures are followed (Rajeshkumar 2017, Soumya et al., 2017, Rajeshkumar et al., 2018, Ponnannikajamidin et al., 2018, Chellakannu et al., 2019)

- DPPH radical assay
- Nitric oxide radical inhibition assay
- Superoxide anion scavenging activity
- Estimation of lipid peroxidation inhibition
- Hydrogen peroxide-scavenging activity:
- ABTS radical scavenging activity
- Hydroxyl radical scavenging assay
- Reducing power

RESULTS AND DISCUSSIONS

Screening of Phytochemicals Constituents

The powdered leaves of P. amboinicus extracted with an aqueous solvent. The resultant extract was dried in the air until the constant weight of the plant extract was obtained. The plant extract was then performed for the phytochemical characteristics to the identification of various phytochemical constituents.

The plants may be consisting of many chemical constituents like alkaloids, glycosides, carbohydrates, proteins, steroids, tannins, saponins, flavonoidsetc. These chemical constituents are called secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of these secondary metabolites in the aqueous extract was subjected to colored reactions of chemical tests. The preliminary phytochemical analysis of an aqueous extract of P. amboinicus leaves revealed the presence of alkaloids, tannins, flavonoids, sugars, phenol, terpenoids and proteins and absence of saponins, glycosides, triterpenoids, and proteins.

Table 1: Phytochemical Constituents of Aqueous Extract of P. Ambonicus Leaves

Compounds	Aqueous extract
Colour of the extract	Yellowish green
Alkaloids	+
Tannins	+
Saponins	+
Glycosides	+
Flavonoids	+
Triterpenoids	-
Sugars	-
Phenol	+
Steroids	+
Terpenoids	+
Proteins	-

+ = Present, -= Absent

Free Radical Scavenging Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of aqueous extract of P. amboinicus leaves exhibited a significant dose-dependent i.e. concentration of plant extract between 2 -10 µg/ml. IC50 values calculated as 50% of inhibition by plant extract concentration. The results of DPPH inhibition by aqueous extract of the plant are shown in Figure 2 and 3.

The 50% of radical scavenging activity was observed at 46.27 µg/ml concentration of aqueous extract. The IC 50 value of the extract was highly significant than the standard (60.13 µg/ml)

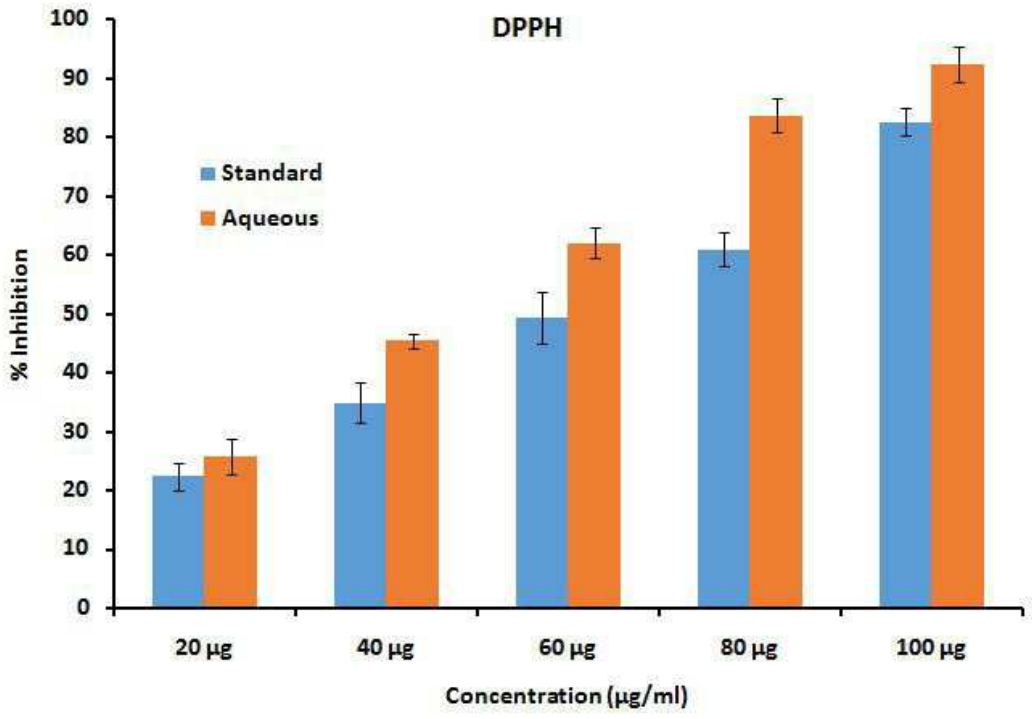

Figure 2: DPPH Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

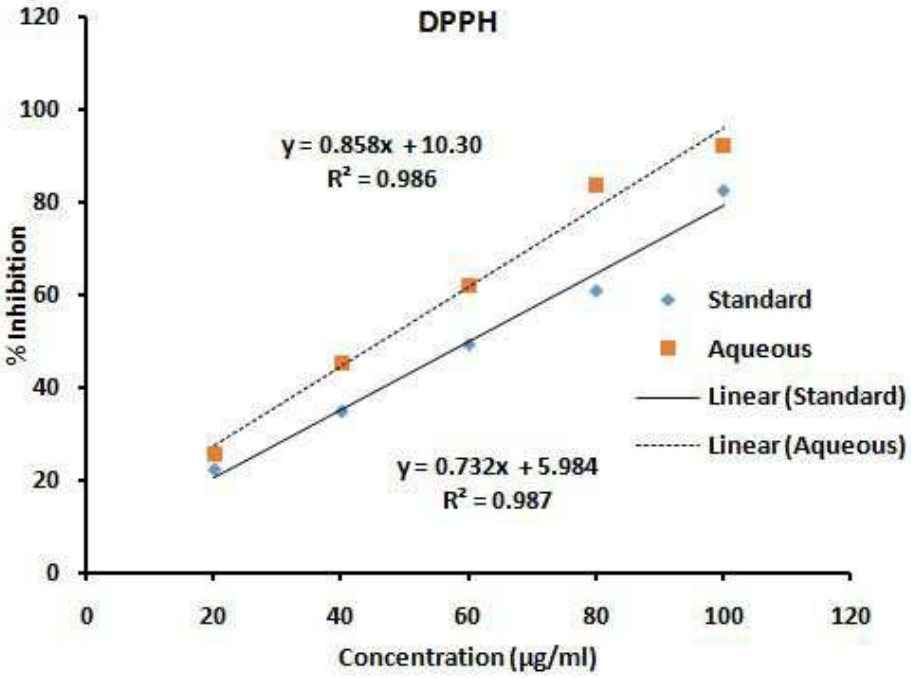

Figure 3: Linear Expression of DPPH Scavenging Activity using the Aqueous Extract of P. Amboinicus Leaves

Nitric Oxide Radical Inhibition Assay

The nitric oxide radical scavenging activity of plant extract was increased while increasing the plant extracts concentration in a dose-dependent manner (Figure 4 and 5). The IC50 value of the aqueous extract was 53.78 µg/ml which was similar to that of standard (63.79 µg/ml). These results showed that aqueous extract of P. amboinicus leaves is known to be an excellent antioxidant agent.

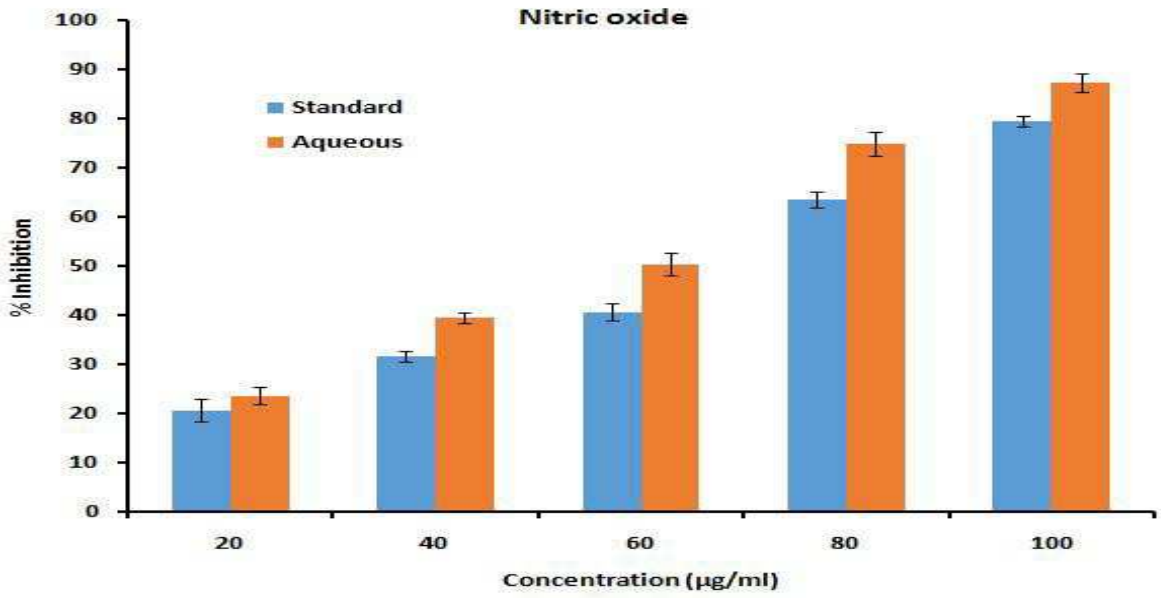

Figure 4: Effect of P. Amboinicus Extract and Standard Ascorbic Acid on Scavenging of Nitric Oxide Radical

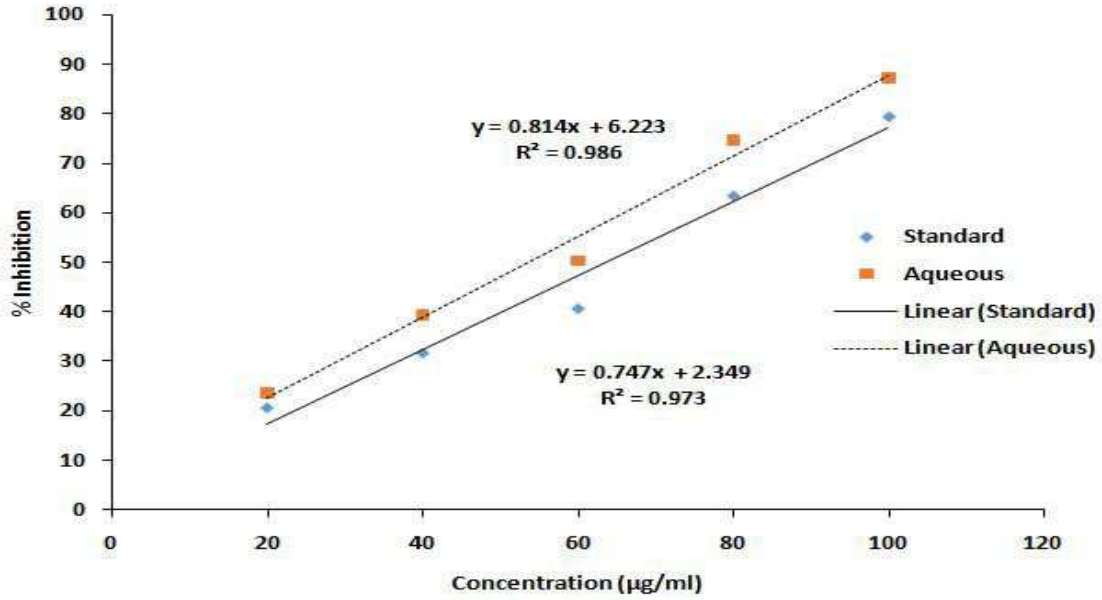

Figure 5: Effect of P. Amboinicus Extract on Nitric Oxide Radical Inhibition Assay

Superoxide Anion Scavenging Activity

Superoxide anion free radical is a highly toxic radical that attacks a number of biological molecules. The superoxide anion derived from dissolved oxygen by Phenazine met hosulphate/NADH coupling reaction reduces nitroblue tetrazolium. Theresultsof super oxide anion scavenging activity o f aqueous extract of P. amboinicus leaves presented in Figure 4. The decrease the absorbance at 560nm with the plant extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in Figure 6 and 7, the plant extract, as well as ascorbic acid, showed the scavenging activity; IC50values, 53.19 µg/ml and 66.22 µg/ml, respectively. P. amboinicus leaf extract efficiently scavenges the superoxide anion than the standards.

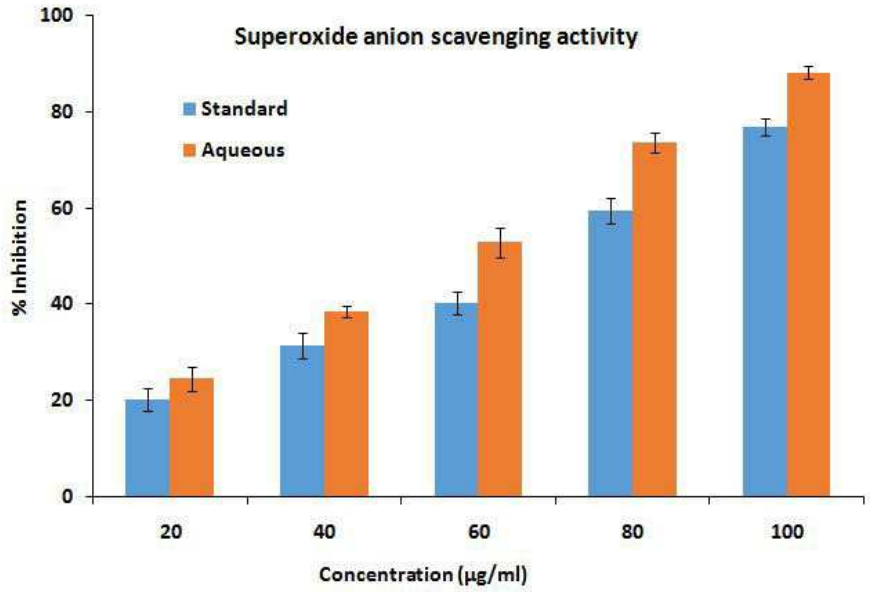

Figure 6: Superoxide Anion scavenging Activity of P. Amboinicus Extract

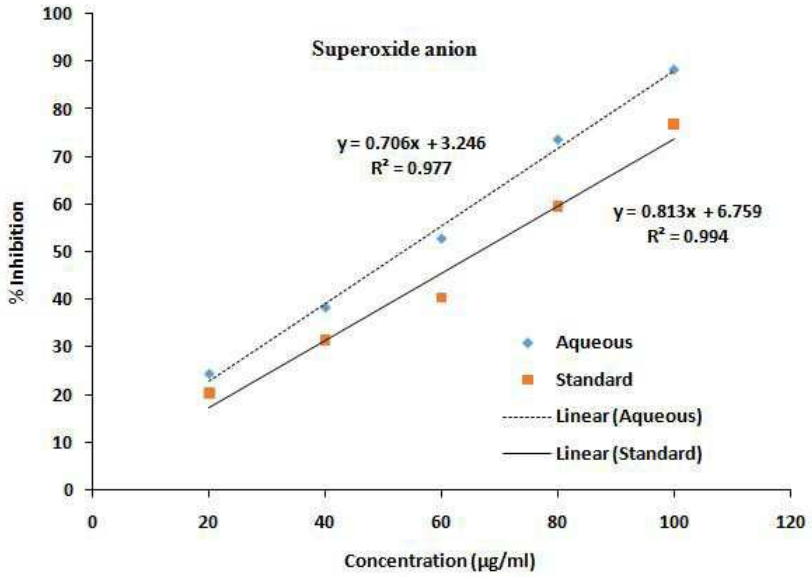

Figure 7: Linear Regression of Super Oxide Anion Scavenging Activity using the Aqueous Extract of P. Amboinicus Leaves

Lipid Peroxidation Assay

Lipid peroxidation inhibition assay was determined by using plant extract which compared with standard. Figure 8 and 9 shows that the extracts have strong inhibiting activityin controlling lipid peroxidation. The scavenging activity was increased as increasing the concentration of plant extract from 20-100µg/ml. The IC50 value was observed at26.7µg/ml had significantly 50 % inhibited lipid peroxidation than the standard.

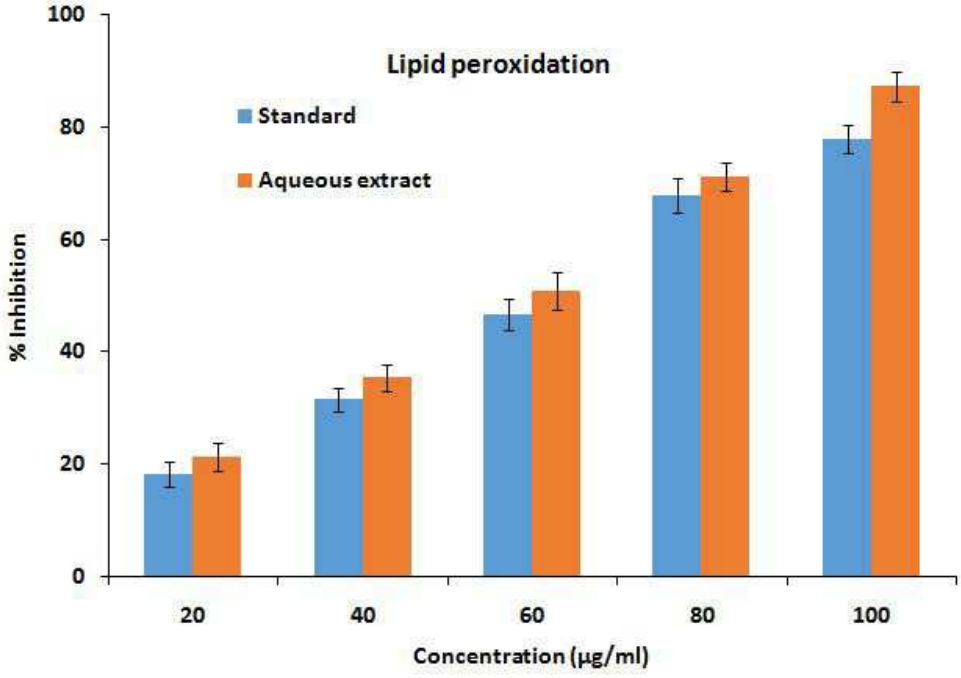

Figure 8: Lipid Peroxidation Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

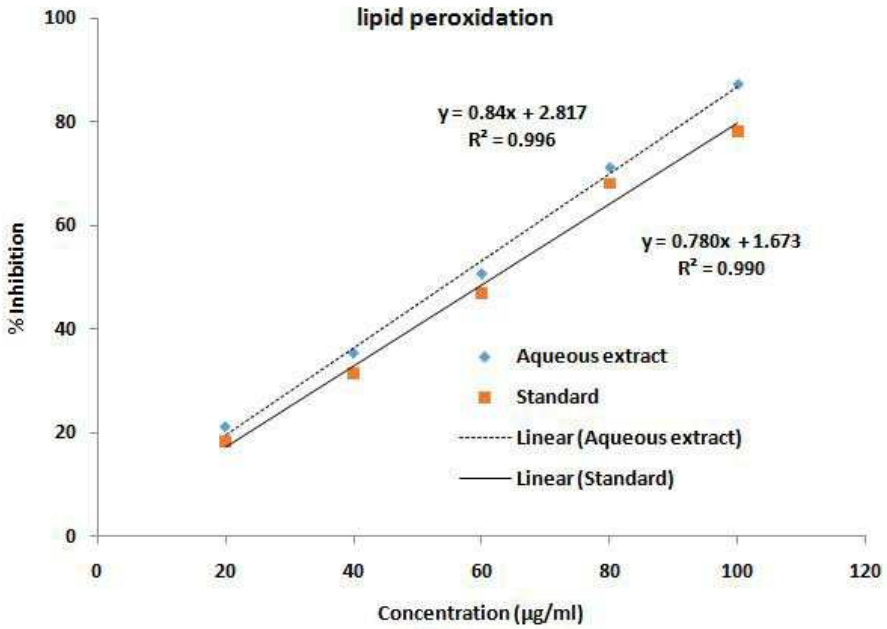

Figure 9: Linear Regression Analysis of Lipid Peroxidation Assay of Aqueous Extract of P. Amboinicus

Hydrogen Peroxide-scavenging Activity

The scavenging of hydrogen peroxide in aqueous extract of P. amboinicus and BHT was analyzed shown in Figure 10 and 11. BHT is a reference standard compound. The activity was shown to be 50 % at a concentration of 44.41µg/ml and 47.75µg/ml for plant extract and standard, respectively. Thus the results show P. amboinicus leaves exhibit scavenging activity which converts hydrogen peroxide into water by donating electron donor.

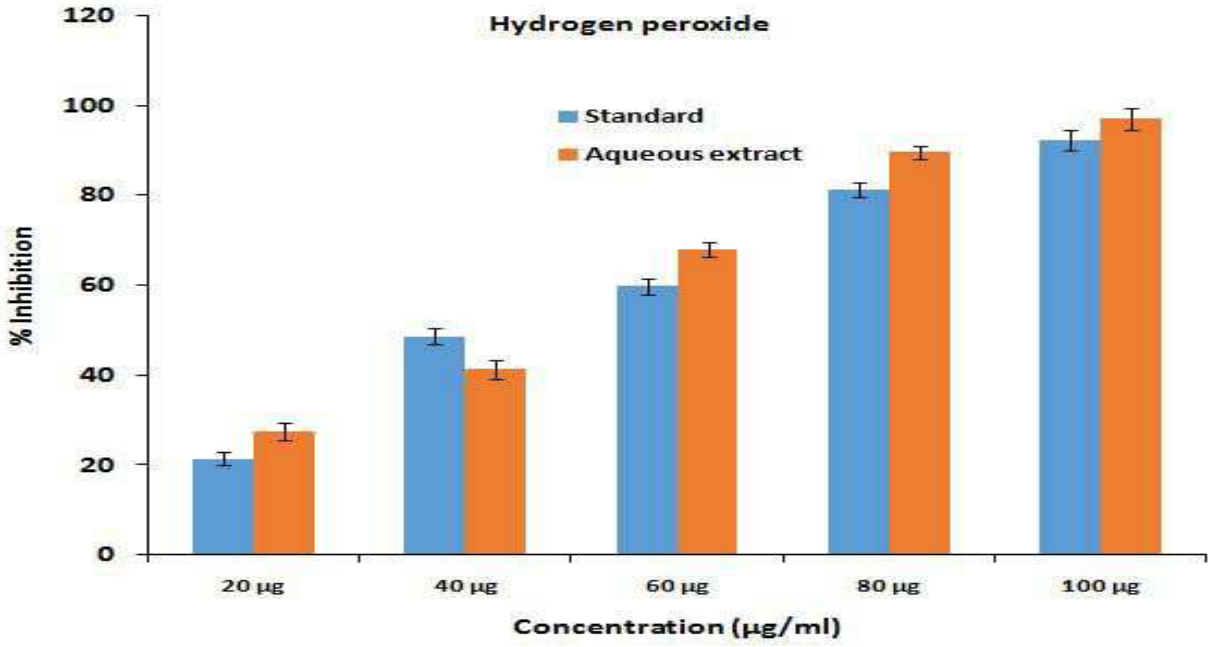

Figure 10: Hydrogen Peroxide Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

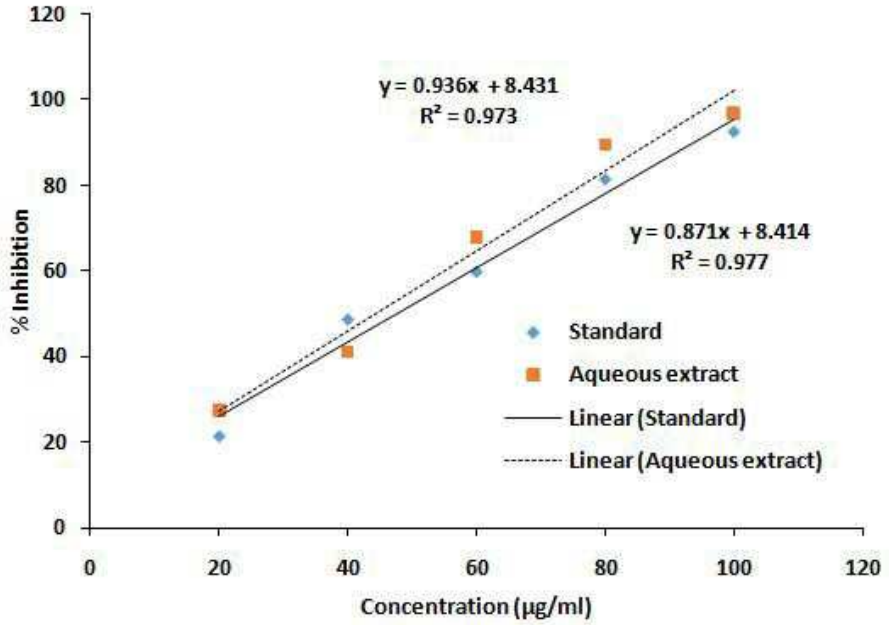

Figure 11: Linear Regression of Hydrogen Peroxide Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

ABTS Radical Scavenging Activity

ABTs radical activity of plant extract was increased in a dose-response manner from 25.75% to 91.087% at a concentration of 20□100 µg/ml (Table 7). The IC50 value for the plant extract and the standard vitamin C is found to be 45.57 and 63.09 µg/ml respectively (Figure 12 and 13). This result show plant extract of P. ambonicus have the ability to scavenge the free ABTS radicals generated by the reaction between 2,2'□azinobis (3□ethylbenzothiazolin□6□sulphonic acid) (ABTS) and potassium persulfate (Leong and Shui

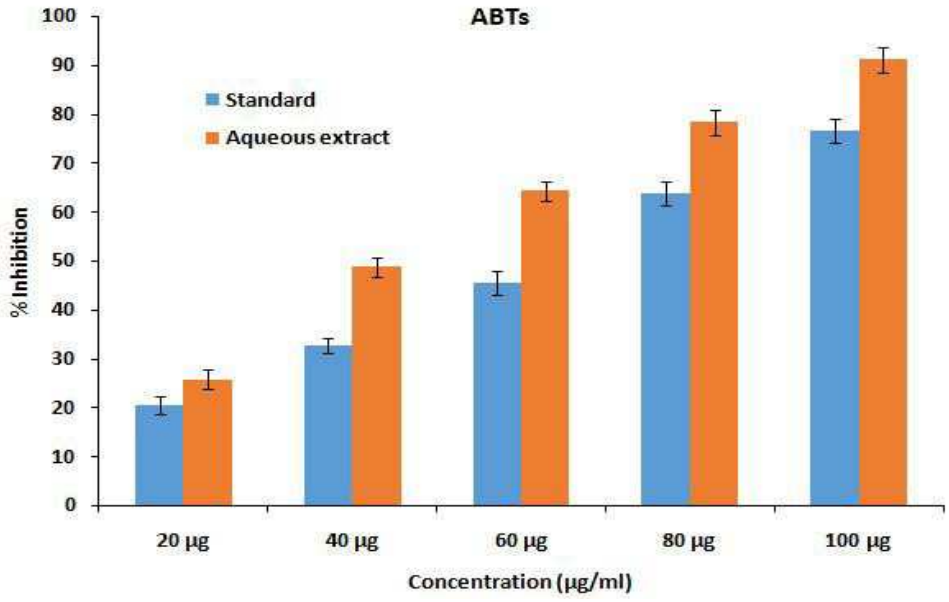

Figure 12: ABTs Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

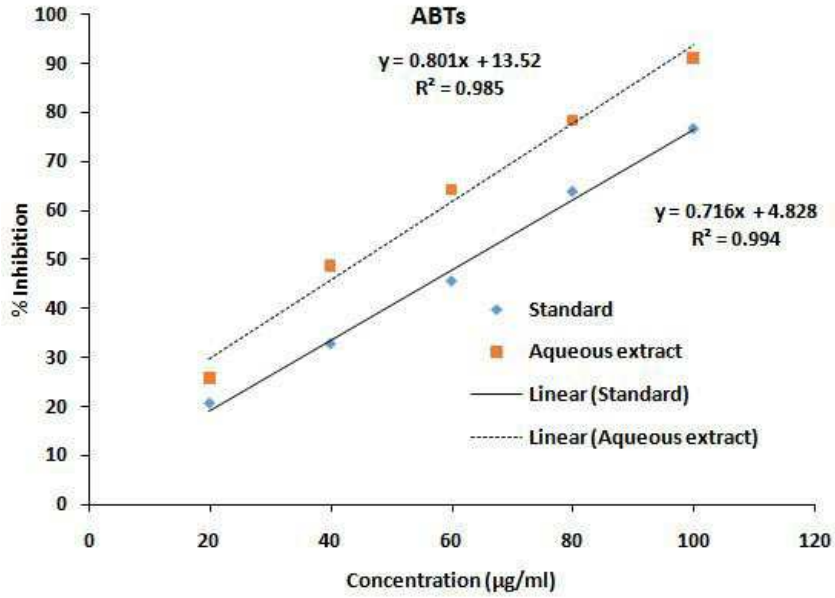

Figure 13: Linear Regression Analysis of ABTS Scavenging Activity of Aqueous Extract of *P.* Amboinicus Leaves

Hydroxyl Radical Scavenging Assay

Figure 14 and 15 showed the aqueous extract of P. amboinicus plant leaves have high inhibition activity against hydroxyl radical. The scavenging activity of plant extract against hydroxyl radical was observed by deoxyribose assay in a concentration-dependent manner. It showed hydroxyl radical scavenging activity with about 50 % at a concentration of 54.63 µg/ml. The results are shown in Table 8, the concentrations of 50% inhibition were found to be 5 4. 6 3 µg/ml and 7 0. 5 2 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

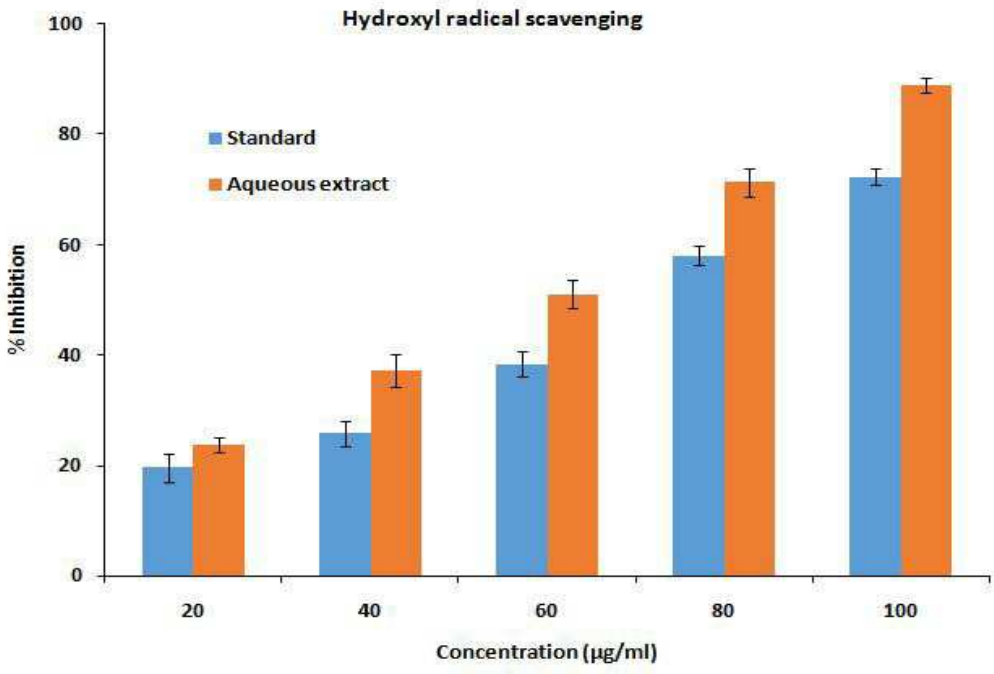

Figure 14: Hydroxyl Radical Scavenging Assay of Aqueous Extract of P. Amboinicus

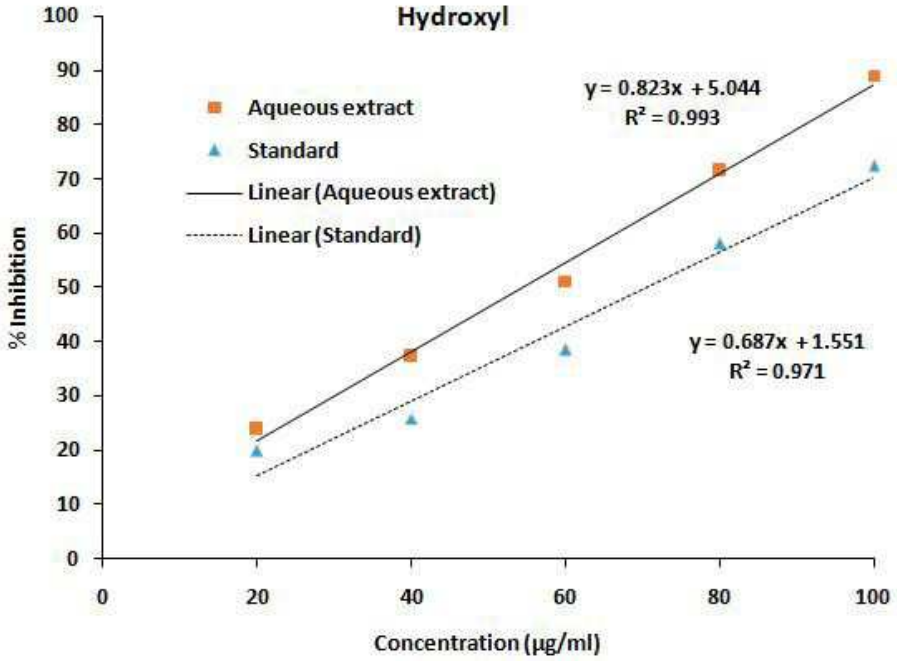

Figure 15: Hydroxyl Radical Scavenging Activity of Aqueous Extract of P. Amboinicus Leaves

Reducing Power

The reducing power of plant extract was analyzed due to electron donating ability (Chellakannu et al., 2019). Figure 16 shows the reducing capacity of the plant extract compare dwith butylated hydroxyl toluene. The reducing power of extract of aqueous extract of P. amboinicus was very effective and the activity was increased with increased concentration of sample (Table 9). In the reaction mixture plant extract would reduce Fe3+ to Fe2+ by donating hydrogen. Thus aqueous extract of P. amboinicus exhibits excellent reducing activity.

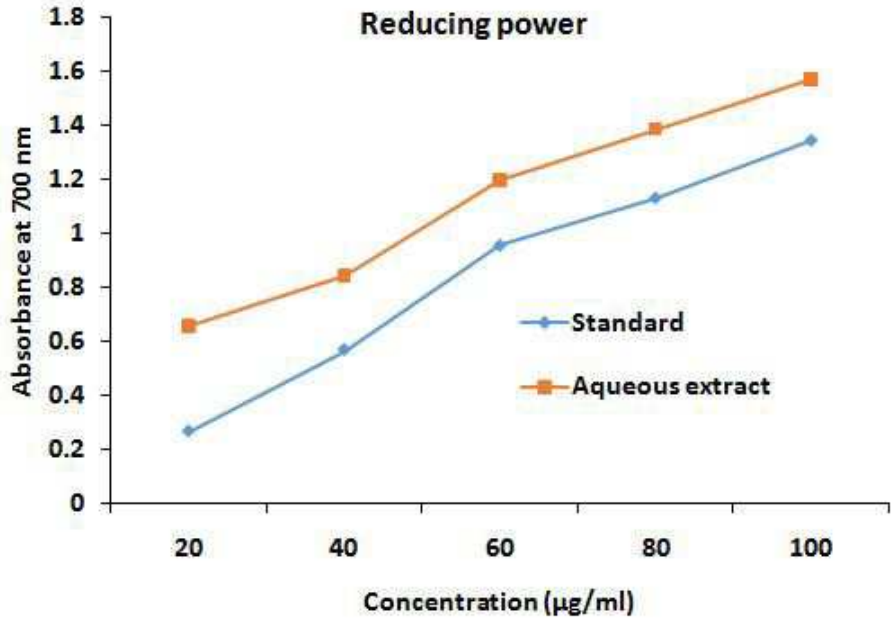

Figure 16: Reducing the Power of Aqueous Extract of P. Amboinicus

Table 9: Reducing the Power of Aqueous Extract of P. Amboinicus Leaves

Concentration (ug/ml)	Standard	Aqueous Extract
20	0.265	0.654
40	0.568	0.843
60	0.956	1.195
80	1.132	1.387
100	1.346	1.572

CONCLUSIONS

In this study, an attempt to find the free radical scavenging activity of aqueous extract of P. amboinicus leaves was the focus of this investigation. Further screening work is required to isolate bioactive metabolites from the crude extracts of this plant. Preliminary phytochemical screening confirms that P. amboinicus contain alkaloids, tannins phenol, glycosides, and flavonoids. Since the compounds with reducing power indicate that they are electron /or hydrogen donors can reduce the oxidized intermediates of lipid peroxidation, therefore the DPPH and FRAP assay were realized P. amboinicus possess a strong scavenging activity due to the presence of a large amount of flavonoids, alkaloids, and Tannins.

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