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COMPREHENSIVE INVESTIGATION ON THE FREE RADICAL SCAVENGING ABILITY OF DIFFERENT PARTS OF PANDANUS UNIPAPILLATUS DENNST. FROM SOUTH INDIA

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ABSTRACT

The present investigation deals with the comparative screening for freeradical scavenging activity of variety of extracts of different plant parts of *Pandanus unipapillatus Dennst.* All the plant parts (root, leaf and stem) were extracted separately using organic solvents with varying polarity. The assays were carried out using nitric oxide scavenging, hydroxyl radical scavenging, superoxide radical scavenging, DPPH methods, total Flavonoids and phenolics. The current investigation revealed that the stem of the plant possesses comparatively higher activity than leaf and root. High amount of flavonoid and phenolic content were found in the stem than that of other parts (leaf, root). The current aim was to identify the most potent part of the plant so that further efforts can be initiated for isolating biologically active molecules from the specified part.

Key words: *Pandanus unipapillatus Dennst. DPPH.* Nitric oxide scavenging., Hydroxyl radical scavenging. Superoxide radical. total flavonoids.

INTRODUCTION

Free radicals are continuously produced in the human body during the normal use of oxygen such as respiration and some cell mediated immune functions. A well dynamic balance between the amount of free radicals generated in the body and antioxidants to scavenge them and protect the body against harmful effects(*Shirwaiker et al.,2006).*The reactive oxygen species (ROS) including superoxide anionic radical(o)2,hydrogen peroxide(H2O2) and hydroxyl radicals are implemented in oxidative damage to various cellular

macromolecules.oxidative stress plays important role in the pathogensis of various diseases such as *Atherosclerosis,alcoholic liver cirrohosis and cancer*.etc.(*Soni et al.,2009*). The antioxidant defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Most living organism have efficient defense systems to prevent themselves against oxidative stress induced by ROS. Recent investigation has shown that the antioxidant properties of plants could be correlated with the oxidative stress defense and different human diseases. In this respect flavonoids and other polyphenolic compounds have received the greatest attention (*Jain et al, .2009*). Flavonoids and Phenolic compounds present in food of plant origin are also potential antioxidants. (Salah N et al., 1995, Van Acker SABE et.al., 1996).

Pandanus unipapillatus Dennst. (Forssk.)Kuntz (*Pandanaceae*), is an attractive screwpine(Local name is "Mundangi","Thazhakaitha"). It is most common in India, the coastal belt and Western Ghats along banks of streams, canals and marshy places. Karnataka, Tamil Nadu and Kerala. Endemic. Shrubs or small trees with few prop roots. Leaves linear-ensiform, up to 200 x 6 cm, margins and midrib prickly. Male inflorescence terminal, spicate. Bracts linear-lanceolate or lanceolate, yellowish, the lower ones flagelliferous. Spikes dense, up to 11 cm long. Stamens many, umbellate on stamenophores; anthers apiculate. Female inflorescence terminal, pedunculate, bracteate. Carpels simple; styles flattened usually bent at right angles, 2-lobed. Fruit (syncarp) single, oblong-rounded, up to 25 cm long, pendulous, orange yellow when ripe; drupesclub-shaped,upto5cmlong,apex.pyramidal (Nicolson et al.interprt.Hort.Malab.306.1988.).

Literature survey revealed that there is a lack of enough scientific reports regarding the antioxidant properties of whole plant *(Pandanus unipapillatus Dennst.)*. Hence the objective of the present investigation on the free radical scavenging ability of different parts (root, leaf and stem) of Pandanus *unipapillatus Dennst.* from South India.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Pandanus unipapillatus Dennst.* were collected during September 2012, from the village areas of Calicut district, north region of Kerala, India. The samples were authenticated from the Department of Taxonomy, Calicut University, Calicut,and Kerala,India. The original specimen of whole plant were Kept on Herbarium sheets

.(CLPO021(Root), CLPO022(Leaf), CLPO023)(Stem). The study was conducted during November 2012 to Januvary 2013.

Preparation of extract

All the plant materials [root, leaf, and stem] were dried in shade and were powdered to mechanical grinder. 10 gm each of powdered plant material were taken separately and its components were extracted individually with hexane, chloroform, Ethyl acetate, methanol, water (100ml) at respective boiling point of extracts. The extracts [Total 15] were then filtered using whattman 1 filterpaper cooled and concentrated to dryness under reduced pressure using a rotary evaporator.

Chemicals

2,2-Diphenyl-1-picrylhidrazyl(DPPH),Gallic acid,methanol,chloroform,Petroleum ether, Folin-Ciocalteu reagent, sodium nitrite, sodium carbonate, phosphate buffer, nitro blue tetrazolium(NBT),EDTA,Riboflavin,Deoxy ribose were purchased from Merck co(Mumbai, India). Sulphanilamide, phosphoric acid and naphthyl ethylene Dihydrochloride, H2O2 ,TBA,TCA were purchased from Sigma Aldrich.

Determination of Total phenolic content

The total phenolic content was determined using Folin–Ciocalteu reagents with analytical grade gallic acid as the standard. 1 mL of extract or standard solution (0 to 500 mg/L) was added to deionized water (10 mL) and Folin–Ciocalteu phenol reagents (1.0 mL). After 5 min, 20% sodium carbonate (2.0 mL) was added to the mixture. After being kept in total darkness for 1h. A blue colour was developed in each tube, because the phenols undergo a complex redox rection with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium which resulted in molybdenum blue. A blue colored the absorbance of the reaction mixture was measured at 650 nm using a spectrophotometer . Amounts of TP were calculated using gallic acid calibration curve. The results were expressed as gallic acid mg /g of dry plant matter (Kim et al., 2003).

Determination of Total Flavonoids

The TF were measured by taken from the methodology of chang et al (2002).Methanolic extract(3g)of each extracts were mixed with 3 ml of methanol,0.2 ml of 10% $AlCl_3$,0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. After being kept in room temperature in

30 minutes.The absorbence was measured at 415nm.For each sample reading were taken to get the average results. The results were expressed in mg quercetin/g dry weight by comparison with the quercetin standard curve, which was made under the same condition.

***In-vitro* antioxidant assay**

DPPH Radical Scavenging assay

The complementary study for the antioxidant capacity of the different parts was confirmed by the DPPH scavenging assay according to (Singh et al.2002). Different concentrations (50-500µg/ml)of the different extracts and standard trolox are mixed with equal volume of ethanol.Then 50µl of DPPH solution(1mM)was pipetted in to the previous mixture and stirred thoroughly.The resulting solution was kept standing for 2 minutes before the optical density(OD)was measured at λ-517.Mesurement was repeated with remaining sets.The percentage radical scavenging activity was calculated from the following formula.

Percentage of scavenging DPPH= [(A0-A1)/A0]*100

Where,A0 was absorbence of the control and A1 was the absorbence in the presence of samples and standard.

Superoxide radical scavenging assay

Assay for superoxide radical scavenging the activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generation in riboflavin-light-NBT system(Ravisankara et al.,2002).The reaction mixture contain 50ml phosphate buffer(PH.7.60,20µg riboflavin,12 mM EDTA,NBT 0.1 µg/3ml added on that sequence). The reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 150 sec.immediately after illumination, the absorbence was measured at 590nm and EC50 was calculated methanol was used for blank reading. Meaurements of superoxide anion scavenging activities of samples and standard Gallic acid were done based on the reduction of NBT according to a previously described method. Superoxide radical is generated by a non-enzymatic system of phenazene methosulphate nicotinamide; adenine dinuclotide (PMS/NADH).These radicals reduce nitroblue tetrazolium (NBT) in to a purple coloured formation. Which was measured spectrometrically at λ=562nm. All test were performed 6 times. The percentage of inhibition of superoxide anion generation was calculated using the following formula.

Percentage of inhibition= [(A0-A1)/A0]*100

Where A0 was the absorbence of the control and A1 was the absorbence in the presence of the samples and standard.

Hydroxyl radical scavenging assay

The scavenging capacity for hydroxyl radical was measured according to the modified method(Rajeswary et al.2005).The assay was performed by adding 0.1ml Deoxyribose,1.0ml of test solution(5-100 µg ml-1) dissolved in distillwater,0.33ml of phosphate buffer(50Mm,PH-7.4) and 0.1ml of ascorbic acid in sequence.The mixture was then incubated at 370c for 1h. a small portion of the incubated mixture was mixed with 1.oml of 10%TCA and 10ml of 5% TBA to develop the pink chromogen.The optical density was measured at 532nm.The scavenging assay for hydroxyl radical was performed by a standard method.hydroxyl radical was generated by the fenton reaction using a Fe3-ascorbate-EDTA-H2O2 system.The assay quantifies the 2-Deoxyribose degradation product by its condensation with TBA. All test were carried out 6 times.Ascorbic acid, a classical OH scavengers was used as a standard compound. Percent inhibition was evaluated by the following equation.

Percentage of inhibition= [(A0-A1)/A0]*100

Where A0 was the absorbance of the control and A1 was the absorbance in the presence of samples and standard. Ascorbic acid was used as standard.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured by the spectroscopic method (Madan et al,2005).Sodium nitroprusside (5mmol) in phosphate buffered saline was mixed with a control without test compound, but with an equivalent amount of methanol. Test solution of different concentration (5-100µg/ml-1) were dissolved in methanol and incubated at 25oc for 150 minutes. After incubation 1.5 mlof incubated solutionwas diluted 1.5ml of Griess reagent (1%-Sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene Dihydrochloride).The absorbence of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthyl ethylene diamine Dihydrochloride was measured at 546nm. Sodium nitroprusside (SNP) gives rise to nitric oxide that under interaction with oxygen produce nitrite ion measured by Greiss Illosvoy reaction. All test

were performed 6 times.Ascorbic acid was used as standard. The percentage inhibition of nitric oxide radical generation was calculated using the following the formula.

Percentage of inhibition= [(A0-A1)/A0]*100

Where A0 was the absorbence of the control and A1 was the absorbence in the presence of the samples and standard. All the solutions were made in triplicate and the average reading were taken for the calculation of the percentage inhibition.

RESULTS

In the present study different solvent extracts of different parts of *Pandanus unipapillatus Dennst.* were studied for its ability to scavenge the oxidants using various in-vitro methods, so as to find out the most potent part of the plant.

Total phenolics and Flavonoids

Flavonoid and Phenolic compounds were found to be 316.7 µg of quercetin equivalents (QE) per mg and 515.13 µg of Gallic acid equivalents (GAE) per mg of the methanolic extract of the stem respectively. The data will be proved the methanolic extract of stem showed high amount of flavonoid and Phenolic content compare than other parts of the plant (root, leaf). The total amount of Flavonoids and phenolic content of methanolic extract of whole plant (root, leaf and stem) of *Pandanus unipapillatus* was summarized in Table 5.

Inhibition of DPPH radicals

The potential decrease in the colour of DPPH radical is due to the scavenging ability of different extracts of *Pandanus unipapillatus Dennst.* (Hexane, chloroform, Ethylacetate, Methanol, water) and Gallic acid (standard). As evident in the table. 1 the Ic50 value of the samples are as in the following the order root <leaf< stem. The positive control used is the gallic acid. In DPPH assay, the IC_{50} of the methanolic extract of stem shown high reducing capacity of free radicals

(104µg/ml).The significant freeradical scavenging activity of methanolic extract of stem and standard reference(Gallic acid) 85% and 64% of inhibition respectively at 100µg/ml^{-1}.(Fig:1).

Table: 1 DPPH Assay of different parts of *Pandanus unipapillatus Dennst.*

EC 50	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Root	188µg/ml	223 µg/ml	234 µg/ml	354µg/ml	298 µg/ml
Stem	**155µg/ml**	**180µg/ml**	**214 µg/ml**	**104µg/ml**	**208µg/ml**
Leaf	182µg/ml	218 µg/ml	295 µg/ml	285 µg/ml	256µg/ml

Hydroxyl radical scavenging assay

This assay shows the abilities of the extracts to scavenge hydroxyl radical. As shown in the table 2. The stem possesses maximum ability to eliminate the hydroxyl ions. Among the various extracts of the stem the methanolic extract (198 µg/ml) showed comparatively more activity than the other extracts. The positive control used is the Ascorbic acid.The percentage of inhibition of methanolic extract of stem and standard(Ascorbic acid) in hydroxyl radical being 52% and 43.7% respectively at 100µg/ml^{-1}.(Fig:2).

Table: 2 Hydoxyl scavenging activity of different parts of Pandanus unipapillatus Dennst.

EC 50	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Root	306µg/ml	309 µg/ml	451 µg/ml	309µg/ml	359 µg/ml
Stem	**206µg/ml**	**201µg/ml**	**203 µg/ml**	**198µg/ml**	**208 µg/ml**
Leaf	276µg/ml	321µg/ml	298µg/ml	256 µg/ml	218µg/ml

Superoxide radical scavenging assay

Table 3 shows the abilities of the 3 different extracts (Root,leaf,stem) of the plant to quench superoxide radicals in the PMS-NADH reaction mixture .the IC 50 values shows that the stems(methanolic extract) is having the maximum capacity to scavenge the superoxide(191 µg/ml). The positive control used is the Gallic acid.The percentage of inhibition of metanolic extact of stem and gallic acid (standard reference) being 60.1% and 57.5% respectively at 100µg/ml^{-1}. (Fig:2).

Table:3 Superoxide Scavenging activity of the different extracts of various plant parts of *Pandanus unipapillatus Dennst.*

EC 50	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Root	305µg/ml	452µg/ml	305µg/ml	269 µg/ml	265 µg/ml
Stem	**284 µg/ml**	**302 µg/ml**	**206µg/ml**	**191 µg/ml**	**206µg/ml**
Leaf	425µg/ml	398 µg/ml	298 µg/ml	247 µg/ml	299 µg/ml

Nitric oxide scavenging assay

As evident from Table 4 the extracts of root, leaf, stem of the plant also showed Nitric oxide scavenging activity, but when looking comparatively the stem(Methanolic)extract showed significant higher activity than the two other parts for all the extracts. Among the five different extracts with varying polarity the methanolic extract of the stem showed higher activity and IC_{50} value is 147 µg/ml. The positive control used is the Ascorbic acid. There was a maximum inhibition of metanolic extract of stem and standard (Ascorbic acid) is 60% and 44% respectively at 100 $\mu g/ml^{-1}$. (fig.4).

Table: 4 EC 50 Values of the Nitric oxide scavenging activity of *Pandanus unipapillatus*

EC 50	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Root	181 µg/ml	211 µg/ml	242 µg/ml	456 µg/ml	201 µg/ml
Stem	**201 µg/ml**	**204 µg/ml**	**151 µg/ml**	**147 µg/ml**	**204µg/ml**
Leaf	266µg/ml	245 µg/ml	176 µg/ml	233 µg/ml	237 µg/ml

Table:5 Total Phenolics and flavanoides in the studied different parts of *Pandanus unipapillatus*

Different parts of Pandanus unipapillatus	Total phenolics (mg *GAE/1 mg DW)	Total flavanoids (mg *QE/1mg *DW)
Stem	515.13 GAE mg g^{-1})	316.7 QE mg g^{-1}
Root	132.4 GAE mg g^{-1})	107.92 QE mg g^{-1}
Leaf	456.3 GAE mg g^{-1})	296.56 QE mg g^{-1}

*GAE- Gallic acid equivalent *QE- Quercetin equivalent *DW- Dry Weight of materials

FIG:1 DPPH SCAVENGING ACTIVITY OF MEOH EXTRACT OF STEM OF PANDANUS UNIPAPILLATUS (STANDARD-GALLIC ACID)

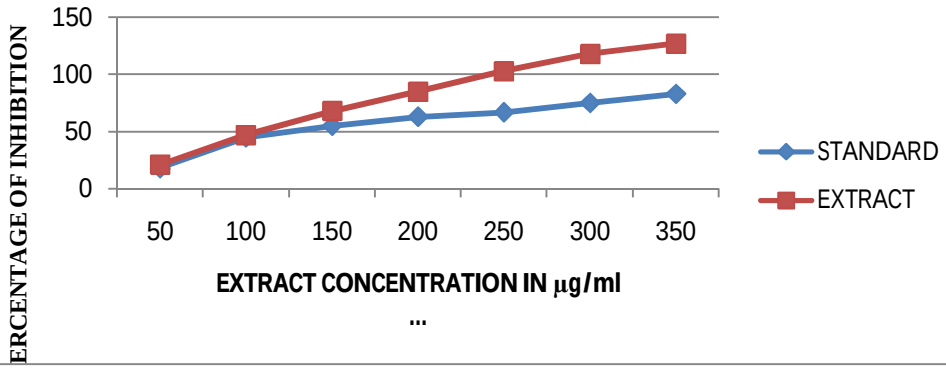


FIG:2 HYDROXYL RADICAL SCVENGING ACTIVITY OF MEOH EXTRACT OF STEM OF PANDANUS UNIPAPILLATUS (STANDARD-ASCORBIC ACID)

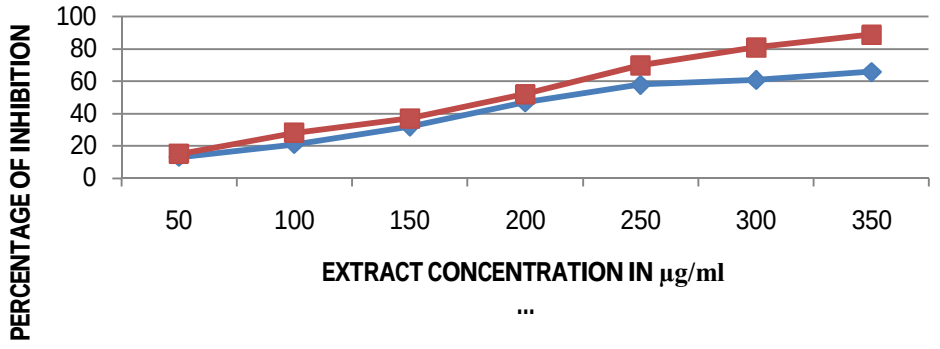
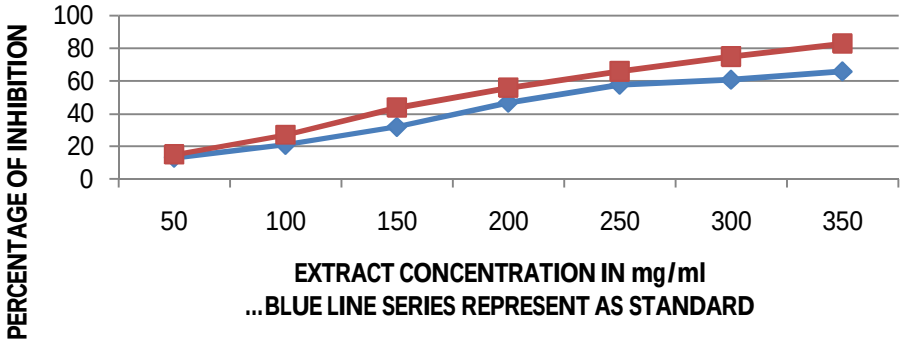


FIG:3 NO_2 RADICAL SCVENGING ACTIVITY OF ETHYL ACETATE EXTRACT OF LEAF OF PANDANUS UNIPAPILLATUS (STANDARD-ASCORBIC ACID)



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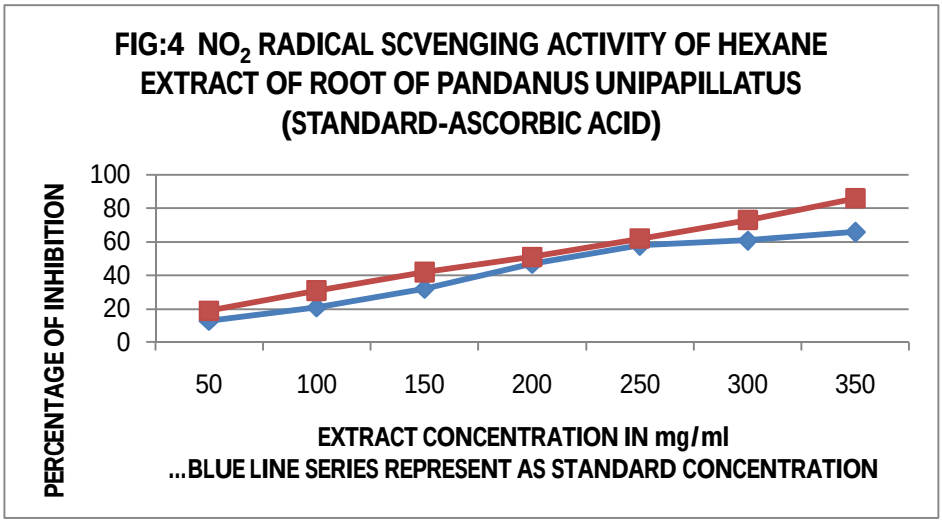

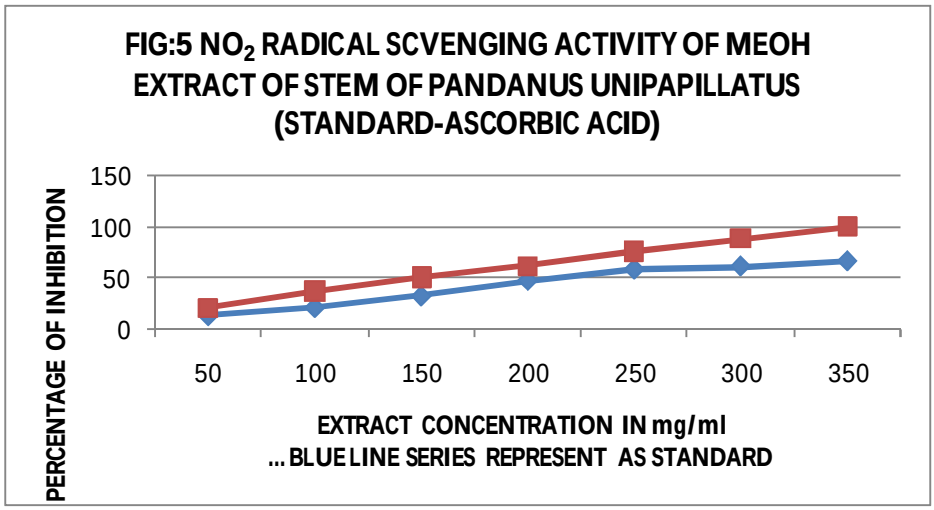

Discussion

Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidized chain reaction. The antioxidative system protects the organism against ROS induced oxidative damage. There are restrictions on the use of synthetic antioxidants, because there are suspected to be carcinogenic (Govindarajan et al, 2003).There for, natural antioxidants have gained importance. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities. The Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical activity. (Christensen Lars P; 1999).

The DPPH is a stable free radical at room temperature and accept an electron or hydrogen radical to become unstable diamagnetic molecules and will make hazardous affects on the

metabolism (Blois, 1958). There was moderate inhibition of the superoxide radical with the maximum inhibition being 60.1% of 1 µg/ml^{-1} extract concentration superoxide anion is oxygen centered radical with selective reactivity. This species is produced by a number of enzymes systems in auto-oxidation reaction and by non-enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complex such as cytochrome (Gulcin et al., 2003). The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids, proteins. The inhibition of free radicals mediated deoxy ribose damage was assessed by means of Iron (ii) dependent DNA damage assay, which showed significant results (Jornot et al.,1998).

Every assay was performed in triplicates for the confirmation and the average values were taken for calculating the EC50 values. From all these methods of antioxidant studies in different parts of the plant (leaf, root and stem), the methanolic extract of stem by DPPH method will be shown high activity of free radical scavenging properties. Literature showed that no information exists about the antioxidant studies of *Pandanus unipapillatus Dennst.* (whole plant) as a source of natural antioxidant.There for the main objectives of the study were to determine *Pandanus unipapillatus Dennst.* As a source of natural antioxidant using different extracting solvents to evaluate their antioxidant capacities.

CONCLUSION

Different plant parts of Pandanus unipapillatus Dennst. Demonstrates significant activity of antioxidant, reducing power and scavenging. From the comparative antioxidant studies which we carried out on different parts (root, leaf and stem) of Pandanus unipapillatus Dennst. The stem showed the most antioxidant activity than the root and leaf. When comparing with all the extracts of the stem the alcoholic extract of the stem possess higher activity in more cases. Thus we conclude that the isolation of the compounds responsible for the antioxidant activity has to be taken up which may result in the identification of new anti-oxidant compounds from the stem of the plant.

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