ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF PANDANUS FASCICULARIS Lam.

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Summary

Plants are widely used in traditional and folklore medicine. Use of medicinal plants and their products are almost doubled over the last decade in developing countries and the present trend of wide spread interest in alternative therapies is well known. *Pandanus fascicularis* is used in traditional systems of medicine to treat varying conditions like rheumatism, fever, headache, earache and also used as antispasmodic. No evidence of scientific study is available on this plant. So the present study is aimed at investigating the invitro antioxidant activity of methanolic extract of *Pandanus fascicularis*. Present study is aimed at investigating the invitro antioxidant activity of methanolic extracts of leaves of *Pandanus fascicularis*(MEPF) by four different in-vitro methods. The lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances (TBARS) in different concentrations of MEPF on normal rat liver homogenates. The reduced glutathione (GSH) was assayed in liver homogenates of different concentrations of MEPF using the method of Ellman et.al. The nitric oxide (NO) scavenging activity and 1-1 Diphenyl,2-Picryl hydrazyl (DPPH) radical scavenging activity was measured using the methods of Sreejayan et.al and Shimada et.al. respectively using spectrophotometer. Vitamin E and normal saline were used as reference standard and control for all four invitro antioxidant measurement assays. The results showed significant antioxidant activity of MEPF in all four in vitro methods used in this study and the IC50,(the half maximal inhibitory concentration, of an inhibitor that is required for 50% inhibition of antioxidant activity) of MEPF was comparable to that of vitamin E, the reference standard compound used in this study.
It is concluded that the methanolic extract of leaves of Pandanus fascicularis has significant antioxidant activity.

Key words: *Pandanus fascicularis*, antioxidant action, lipidperoxidation, reduced glutathione nitric oxide scavenging ,diphenyl picryl hydrazyl radical scavenging.

Introduction

Plants are widely used in various traditional and folklore systems of medicine. Consumption of medicinal plants and their products has almost doubled over the last decade in developed countries. At present the trend of wide spread interest in alternative therapies, is well known. With the recent success of many plant derived drugs such as anticancer agent taxel and it's derivatives from *Taxus baccata* and *Taxus brevifolia* and antimalarial, artemisinin from the Chinese wormwood *Artemisia annua,* the interest is growing.

The development of science and phytochemistry rejuvenated the hopes for remedies in chronic diseases and this has generated new enthusiasm in the research work to develop herbal medicine. WHO (World Health Organization) estimated that 80% of the population in developing countries still relies on plant-based medicine for preliminary healthcare. Now efforts are being made to develop herbal medicines in research institutes. Although India has the tradition of alternative therapies like Ayurveda, Siddha and Unani, there are no procedures to test the safety and efficacy of traditional remedies and to standardize their effective cure. For these reasons we should increase our efforts in the area of medicinal plant research and should exploit efficiently for the benefit of humanity.

Pandanus fascicularis Lam. (Synonyms: Pandanus tectorius, Pandanus odoratissimus Roxb, Family: Pandanaceae). Vernacular names [1, 2]: Sanskrit- ketaki, Hindi-Kura, Kewda, Ketki, Gagandhul, Kannada-Tale mara, English-Screw pine) is distributed widely in coastal regions of Indian subcontinent and Andaman Islands. The plant is a branched palm like shrub, up to 1-3 m high, rarely erect, stem supported by aerial roots, leaves glaucous-green, 0.9-1.5m, ensiform, long lanceolate, acuminate with three rows of prickles each on the margins and on midrib beneath, spinescent. Male flowers in spikes enclosed in large, white fragrant spathes, female flowers in solitary spadix. Syncarpium yellow or red, drups numerous, each consisting of 5-12 carpels; each carpel 5-12.5 cm long, turbinate and angular20. Propagation is by seeds and vegetative method. Leaves, flowers, roots, fruits, spadices, bracts are used in leprosy, smallpox, syphilis, scabies, pain, heat of body, diseases of heart and brain and leucoderma[21]. The tender floral leaves are used to flavour cream, rice (giving a flavour similar to basmati rice) sherbets, jellies and sometimes curries [2, 3]. Oil from bracts is used for headache and rheumatism and considered as stimulant and anti spasmodic[3]**.** Kewda attar or water prepared by distillation of spadices is used to flavour sweets, syrups and soft drinks[2].The flower is pungent, bitter; improves complexion. The anthers are useful in pruritis, earache, headache, leucoderma, eruptions, and diseases of the blood. Fruit is useful in relieving "vata", "kapha" and urinary discharge and is beneficial in leprosy[3]. A medicinal oil is prepared from the roots is considered as diuretic, depurative and tonic[3]**.** Juice obtained from inflorescence is used for rheumatic arthritis in veterinary medicine[4]. Kewda oil is used in ear ache, head ache, arthritis, debility, depurative, giddiness, laxative, leprosy, rheumatism, small pox and spasms[5].

The principle constituent of kewda oil responsible for the characteristic aroma of spadices is b-phenyl ethyl methyl ether. 2-acetyl –1 pyrroline is a major volatile component in the tender floral leaves or spathes. Blossoms yield 0.1-0.3% essential oil called kewda oil containing benzyl benzoate, benzyl salicylate, benzyl acetate, benzyl alcohol, geraniol, linalool, linalyl acetate, bromostyrene, guaiacol, phenyl ethyl alcohol, and aldehydes. Cirsilineol, n-triacontanol, β-

sitosterol, β-sitostenone, stigmast-4-ene-3,6dione stigmasterol, campesterol, daucosterol, and palmitic acid, stearic acid isolated from rhizomes[2,6,7,8]

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. The greatest disadvantage in the potent synthetic drugs available at present lies in their side effects, toxicity and reappearance of symptoms after discontinuation. Hence search for new anti- rheumatic agents that retain the therapeutic efficacy and devoid of adverse effects are justified.

So *Pandanus fascicularis,* a plant that was traditionally used for rheumatism with no scientific proof claimed as yet on its leaf is investigated in this work. Inflammatory state is associated with free radical formation and cell damage. The present study has been undertaken to investigate and evaluate methanolic extract of leaves of *Pandanus fascicularis* for its free radical scavenging potential. In this study after preliminary phytochemical screening of the extract, its in vitro antioxidant activity were tested out using lipid peroxidation, reduced glutathione assay, nitric oxide and DPPH scavenging activity and compared with that of vitamin E the standard antioxidant compound.

Materials and Methods

Leaves of *Pandanus fascicularis* used for the investigation were collected from the east coast road, Chennai-96, in the month of April 2004. The plant was identified and authenticated by research officer (Pharmacognosy), Central Research Institute (Siddha), Arumbakkam, Chennai-600106.

Preparation of methanol extract :

The leaves of *Pandanus fascicularis* were collected, coarsely powdered and was successively extracted with methanol using Soxhlet extractor. The methanol extract of *Pandanus fascicularis* (MEPF) was dried under reduced pressure using a rotary flash evaporator and it was kept in the refrigeration. The percentage of yield of methanolic extract was 9%.

The methanol extract thus obtained was used for the preliminary phytochemical screening and pharmacological studies such as for in-vitro antioxidant activity. The liver homogenate (3ml) of normal rats was used for invitro lipid peroxidation(LPO) and reduced glutathione(GSH) activity of MEPF after dissolving it in 3ml normal saline in varying concentrations (25µg-800 µg/ml). Lipid peroxidation (LPO) was assayed by estimating thiobarbituric acid reactive substances(TABRS) and reduced glutathione (GSH) was assayed in the liver homogenates of normal rats with different concentrations of methanol extract of *Pandanus fascicularis* (MEPF).The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) , Reference No: IAEC-X-3 / CLBMCP / 2004-2005.

PRELIMINARY PHYTOCHEMICAL SCREENING [9,10]

The methanol extract of *Pandanus fascicularis* leaves was subjected to preliminary phytochemical screening. This extract was tested for the presence of alkaloids, carbohydrates, proteins, steroids,sterols,phenols,flavonoids,tannins,gums.mucilage,glycosides,saponins and terpenes[9,10.].

IN-VITRO ANTI OXIDANT STUDIES:

Lipid peroxidation (LPO)[11]

The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances (TBARS). Different concentrations (25-800 µg/ml) of MEPF were added to the normal rat liver homogenate(3ml). LPO was initiated by adding 100 µl 15 mM ferrous sulphate solution to 3 ml of liver homogenate. After 30 min 0.5ml of this reaction mixture was taken in a tube containing 1.5 ml of 10 % w/v trichloroacetic acid (TCA). After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. The mixture was heated in a boiling water bath at 85 °C for 30 min to complete the reaction. The pink coloured complex formed was measured at 535 nm in a spectrophotometer. Vitamin-E was used as reference standard. The percentage inhibition of LPO was calculated by comparing the results of the test with those of controls not treated with the extracts as per following formula -Percentage inhibition = {(control-test) /(control)} X 100.

Reduced glutathione (GSH) assay[12]:

Liver homogenate(3ml) with different concentrations (25-800 µg/ml) of MEPF was mixed with 0.5 ml of precipitating buffer (5% w/v TCA in 0.1 mM EDTA). The sample was centrifuged at 2000 rpm for 10 min and the supernatant was mixed with 2.5 ml of 0.1 M phosphate buffer (pH 8.0). The colour was developed by adding 100 µl of 0.01% DTNB. Absorbance was noted at 412 nm using UV spectrophotometer. The percentage reduction was calculated by comparing with control. Vitamin-E was used as reference standard.

Nitric oxide (NO) scavenging activity[13]:

Nitric oxide scavenging activity was measured by using spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffer saline was mixed with different concentrations of MEPF (25-800 µg/ml) dissolved in normal saline and incubated at 25°C for 30 min. A control without test compound but with equivalent amount of sodium nitroprusside was taken. After 30 min 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthyl ethylene diamine dihydrochloric acid was measured at 546 nm. Vitamin-E was used as reference standard.

1-1 Diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity[14]:

DPPH scavenging activity was measured by spectrophotometric method. 0.1 mM solution of DPPH was prepared in ethanol and 1 ml of this solution was added to 3 ml of MEPF in normal saline at different concentrations (25-800 µg/ml). Equal amount of normal saline was added to the control. The mixture was shaken well and incubated at room temperature for 30 min. The absorbance was read at 517 nm using a spectrophotometer. Vitamin-E was used as reference standard. All the assays were read at a particular nm using spectrophotometer, UV –1601 Shimadzu

Statistical tests: In in-vitro experimental methods the percentage of inhibition and the IC50 value or the half maximal inhibitory concentration, representing the concentration of an extract that is required to inhibit 50% of oxidant activity were calculated and compared with

that of the standard antioxidant compound vitamin E and the control(normal saline) used in this experiment. Results were presented as mean ± SEM and the correlation coefficient denoted as "r", indicating the potency of the test compound as compared to the standard. The antioxidant activity is considered as significant if the "r" value is >0.5. The 'p' value is not mentioned here (though it is $p<0.001$) since here it indicates the lesser activity than the standard compound used.

Results

Preliminary phytochemical screening:

The results of preliminary phytochemical screening of the methanol extract of *Pandanus fascicularis* leaves showed the presence of alkaloids, carbohydrates, phenols, steroids, sterols, proteins and glycosides.

In vitro antioxidant methods:

Lipid peroxidation:

MEPF inhibited the ferrous sulphate induced lipid peroxidation in a concentration dependent manner. The IC_{50} value of MEPF was found to be 669.23 µg/ml(r= 0.61). The IC_{50} value of vit-E was 411.3 µg/ml (Table - 1). The regression coeifficient "r" is the ratio between vitamin E and MEPF indicating the potency of MEPF as compared to vitamin E.

Reduced glutathione assay.

MEPF inhibited the oxidation of reduced glutathione in a dose dependent manner. The IC_{50} values of MEPF was found to be 697.1µg/ml (r= 0.59). The IC_{50} value of vit-E was 414.25 µg/ml (Table-1).

Table 1: Effect of MEPF on Lipid peroxidation (LPO) and oxidation of GSH in rat Liver homogenate (Mean ± SEM, n=6)

Drug (MEPF/Vit. E) concentration (µg/ml)	Percent (%) inhibition		Percent (%) inhibition	
	Lipidperoxidation(LPO) in liver homogenate		Oxydation of GSH in liver homogenate	
	MEPF	Vit. E	MEPF	Vit. E
25	2.67 ± 0.04	28.31 ± 0.05	4.25 ± 0.14	25.66 ± 0.16
50	10.47 ± 0.02	40.84 ±0.02	12.12 ±0.06	41.86 ± 0.01
100	22.56 ± 0.02	57.54 ±0.02	25.91 ±0.05	55.36 ± 0.08
200	35.45 ± 0.02	67.95 ±0.02	35.91 ±0.57	68.40 ± 0.12
400	44.49 ± 0.01	81.42 ±0.10	43.71 ±0.15	75.75 ± 0.08
800	59.77 ± 0.01	97.25 ±0.03	57.38 ±0.24	96.56 ± 0.12
IC_{50}	669.23± 0.04	411.3± 0.14	697.1 ±0.11	414.25 ±0.15
r=Vit.E/MEPF	0.61		0.59	

Table 2: Effect of MEPF on nitric oxide scavenging activity and free radical scavenging activity by DPPH reduction action (Mean ± SEM, n=6)

Drug (MEPF/Vit. E) Concentration (µg/ml)	Percent (%)Inhibition		Percent (%)Inhibition	
	Nitric oxide scavenging activity		Free radical scavenging activity by DPPH reduction	
	MEPF	Vit. E	MEPF	Vit. E
25	5.3 ± 0.04	24.20 ± 0.16	8.27 ± 0.04	26.30 ± 0.09
50	12.3 ± 0.08	39.46 ± 0.02	17.55 ± 0.05	37.66 ± 0.02
100	20.4 ± 0.06	50.82 ± 0.01	29.35 ± 0.07	47.30 ± 0.03

200	31.8 ±0.06	67.82 ± 0.01	43.03 ±0.04	60.60 ± 0.10
400	43.2 ± 0.08	81.05 ± 0.01	52.49 ± 0.50	79.19 ± 0.08
800	56.1 ± 0.02	96.18 ± 0.01	66.98 ± 0.03	98.77 ± 0.03
IC_{50}	713.01± 0.07	415.89 ±0.02	597.19± 0.50	404.98 ± 0.18
r=Vit.E/MEPF	0.58		0.68	

Discussion

Indigenous drug system can be a source of a variety of new drugs, which can provide relief to pain, fever and inflammation but their claimed reputation has to be verified on a scientific basis. The present investigation revealed that the extract of *Pandanus fascicularis* leaves (MEPF) has a significant antioxidant activity in all the four invitro models used in this study.

Recent studies suggest that inflammation and tissue damage are due to the liberation of free radicals[15]. The free radicals have been implicated in the pathophysiology of various clinical disorders including inflammation, acute hypertension and cancer etc.[16]. Normally endogenous intracellular antioxidants protect the tissue from injury by free radicals[17].Therefore development of antioxidant drug could be beneficial as adjunct to anti-inflammatory therapy. Phytochemical screening revealed the presence of phenols, which could be responsible for its anti-inflammatory and antioxidant activity. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action.

The MEPF showed significant protection against ferrous sulphate induced LPO which could cause by absence of ferryl perferyl complex. It is generally assumed that ability of the plant phenolic compounds to chelate iron in LPO system is very important for their antioxidant property. Therefore an attempt was made to determine the role of iron chelation, since the inhibition of ferrous sulphate induced LPO could also be due to chelation of iron. So it can be concluded from the present study, the extract offers protection against ferrous sulphate induced LPO by either metal chelation or absence of ferryl perferyl complex, which is essential for inhibition of LPO[18]. GSH is a non enzymic mode of defense against the free radicals. Thiols especially cystein and glutathione are important in leukocyte functioning[19].

The study on nitric oxide scavenging demonsrates that the methanol extract of *Pandanus facicularis* is a potent scavenger of nitric oxide. NO generated from sodium nitroprusside reacts with oxygen to form nitrite ions which can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO[13.]

The free radical scavenging activity of the plant extract MEPF was evaluated based on the ability to quench the synthetic DPPH. Because of the odd electrons DPPH shows a strong

absorption band at 517 nm in visible spectrum. As this electron becomes paired off in presence of free radical scavenger, the absorption vanishes and the resulting decolourisation is stoichiometric with respect to the number of electrons taken up[20]. The bleaching of DPPH absorption is representative of the capacity of the test compounds to scavenge free radicals independently. The results revealed that the test compound /extract is an electron donor and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. In the present study it can be concluded that the MEPF has significant antioxidant activity. Since the antioxidants have been demonstrated to be useful in inflammatory disorders[21] the claimed beneficial effects of *Pandanus fascicularis* in traditional medicine in various rheumatic disorders could be due to its antioxidant activity. This enables us for further research to find out the active principle responsible for the antioxidant action and its isolation to be used in rheumatic disorders.

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