Research Article

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITIES OF *CALAMUS VATTAYILA*

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ABSTRACT

The present paper deals with the study of phytochemical screening, anti-arthritic and antioxidant activity of aqueous fruit extract of *Calamus vattayila* Renuka. Phytochemical screening of the extract revealed the presence of tannins, steroids, flavonoids and terpenoids. The total phenolic content of the extract observed was 78.3 mg/g equivalent of gallic acid. The antioxidant activity was studied by using *in vitro* antioxidant models viz., DPPH radical scavenging assay, superoxide anion radical scavenging assay and reducing power assay. The extract showed antioxidant activity by inhibiting DPPH free radicals with IC_{50} value 77 µg/ml. The reducing power ability of the extract in ferric reducing model was dose dependent. The maximum percentage inhibition in superoxide anion radical scavenging assay was found to be 65%. The result of the anti-arthritic assay indicated that concentration dependent protein denaturation by the extract.

Keywords: *Calamus vattayila*, Antioxidant activity, anti-arthritic activity, Phytochemicals.

INTRODUCTION

Free radicals play an important role in the pathogenesis of several human diseases, such as cancer, rheumatoid arthritis, and cardiovascular diseases[1]. Rheumatoid arthritis is an autoimmune disease which shows inflammation of joints, destruction of articular synovial proliferation [2]. The phytochemicals present in fruits, vegetables and natural plant products are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species[3]. The use of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are being restricted due to their side effects such as carcinogenicity [4]. Therefore, there is a dire need to find natural antioxidants for use in foods or medicinal materials to replace the synthetic antioxidants.

The genus *Calamus* comprises about 370 species and is mainly found in the tropical rain forests [5]. *Calamus vattayila* is a perennial high climbing spiny palm with solitary stem. The plant is dioecious, flowering is annual and pleonanthic. The species of *Calamus* are used in Ayurvedic system of medicine for curing various diseases like Cough, Edema, Herpes, Diabetes, Rabies etc [6]. The tender shoots of *C. erectus*, *C. floribundus* and *C. latifolius* are used as vegetable and also as a cure for stomach ulcer and muscular sprain [7].

MATERIALS AND METHODS

Chemicals

Gallic acid, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, folin-ciocalteu reagent, PMS, NADH, bovine serum albumin etc. were purchased from Merck India Ltd., Mumbai and 1,1-diphenyl-2- picryl hydrazyl (DPPH) was obtained from Sigma (St. Louis, USA). All the reagents and solvents used were of analytical grade.

Collection of plant material and extraction

The fruits of *Calamus vattayila* were collected from Sampaje, Kodagu District, Karnataka, India. The collected fruits were washed thoroughly in water and dried. The dried fruits were pulverized in electric grinder. 100g of powder was homogenized with a litre of water and the homogenate was kept in a shaker at 40ºC for 24 hrs and then filtered using whatman

No.1 filter paper. The filtrate was concentrated in a lyophilizer. The extract was stored in a deep freezer until use.

Phytochemical Screening and Evaluation of total phenolic contents

Preliminary phytochemical screening of the extract was carried out according to the method of Khandelwal [8]. Total phenolic content of the extract was determined by using Slinkard and Singleton method [9]. The reaction mixture contains 0.1 ml of extract (1mg/ml) and one ml of Folin-Ciocalteu reagent. After three minutes of incubation 3ml of 2% Na_2Co_3 was added. The mixture was incubated for 2 hrs at room temperature with intermittent shaking. The absorbance was measured at 760nm. The standard graph was prepared using different concentrations of gallic acid. The concentration of total phenolic compounds in a sample was determined as mg of gallic acid equivalent per gram of dry extract.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Kumaran and Karunakaran [4]. Briefly, 0.1 ml of plant extract and standard ascorbic acid solution at different concentrations were mixed with 3 ml of 0.004% DPPH solution. After 30 minutes of incubation in the dark, absorbance was taken at 517 nm. The percentage inhibition was calculated using the following fomula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where 'A_C ' is the absorbance of the control. 'A_S' is the absorbance of the sample.

Reducing power assay

Oyaizu method has been employed to determine reducing power of the extract with slight modification [10]. Different concentrations of plant extract and standard ascorbic acid solutions were mixed with 2.5 ml of phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 minutes. Then, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2.5 ml of upper layer solution was mixed with equal amount of distilled water and 0.5 ml ferric chloride. The absorbance was measured at 700 nm.

Superoxide anion radical scavenging assay

PMS-NADH system was used to generate superoxide anions [11]. Briefly the reaction mixturecontains 1mL of nitro blue tetrazolium, 1mL NADH in 100mM phosphate buffer and 0.1mL of sample solution at different concent rations. The reaction was started by adding 100µl of PMS and incubated at 25°C for 5 minutes.The absorbance of the mixture was measured at 560nm.

Ascorbic acid was used as reference standard. The percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where 'A_C 'is the absorbance of the control. 'A_S 'is the absorbance of the sample.

Anti-arthritic assay

Protein denaturation method has been used to evaluate anti-arthritic activity [12]. The reaction mixture consists of 0.45 ml bovine serum albumin and 0.05 ml extract at different concentrations. Samples were incubated at 37^{o}C for 20 minutes then the temperature was increased to 57^{o}C for 3 minutes. 2.5ml of Phosphate buffer was added to all the samples after incubation. Absorbance was measured at 416 nm. Diclofenac sodium was used as reference drug. The Percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where 'A_C 'is the absorbance of the control. 'A_S ' is the absorbance of the sample.

Statistical analysis

Experimental results were mean ± SD of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value.

RESULTS AND DISCUSSION

Phytochemical Screening and Evaluation of total phenolic contents

The phytochemical screening of the extract showed the presence of tannins, steroids, flavonoids and terpenoids (Table 1). Phenolic compounds are a class of antioxidant agents, which act as free radical scavengers [13]. The total phenolic content of the extract was found to be 78.3 mg/g equivalent of gallic acid. The interests in phenolic compounds have considerably increased in recent years because

of their diverse biological properties. Phenolic compounds are effective hydrogen donors which make them antioxidant [14].

DPPH radical scavenging activity

The strong free radical scavenging effect of extract was confirmed in DPPH assay (Table 2). The IC_{50} value of the extract was found to be 77µg/ml. DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule [15]. Hence, the free radical scavenging capacity of an extract may serve as a significant indicator of its potential antioxidant activity.

Reducing power assay

The result clearly indicated that the reducing power of the extract increased with increasing concentration and is comparable with the standard antioxidant ascorbic acid (Table 3). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [16]. The study showed phenolic compounds present in the extract may act as electron donors and could react with free radicals to convert them in to more stable products and terminate the radical chain reactions.

Superoxide anion radical scavenging assay

The extract showed potent superoxide radical sc avenging activity in a dose dependent manner which is comparable to standard ascorbic acid. Maximum percentage inhibition of the extract was found to be 65% (Table 4). Superoxide anion is a harmful reactive oxyg en species as it damages cellular componen ts in biological systems [17].

Anti- arthritic assay

Denaturation of protein is one of the causes for rheumatoid arthritis [18]. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. In the present investigation extract was evaluated against denaturation of bovine serum albumin protein. The result showed concentration dependent inhibition of protein denaturation by the extract. The maximum inhibition of protein denaturation observed was 56 % (Table 5).

Table 1: Showing phytochemical constituents of ethanolic fruit extract of *Calamus vattayila*

Terpenoids	Saponins	Steroids	Alkaloids	Glycosides	Tanins	Flavanoids
++	--	++	--	--	++	++

++ Presence of constituent
-- Absence of constituent

Table 2: Shows DPPH radical scavenging activity of the ethanolic fruit extract of *Calamus vattayila* and Ascorbic acid

Concentration of the extract and ascorbic acid (µg)	% inhibition of the extract	% inhibition of ascorbic acid
20	12 ± 0.08	92 ± 0.6
40	26 ± 0.9	96 ± 1.2
60	40 ± 1.1	97 ± 1.6
80	52 ± 1.2	98 ± 1.2
100	61 ± 0.7	99 ± 1.6

Table 3: Showing reducing power activity of the ethanolic fruit extract of *Calamus vattayila* and Ascorbic acid

Concentration of the extract and Ascorbic acid (µg)	Reducing property (absorbance) of the extract	Reducing property (absorbance) of the ascorbic acid
20	0.09 ± 0.01	1.1 ± 0.06
40	0.12 ± 0.03	1.7 ± 0.1
60	0.32 ± 0.01	1.9 ± 0.2
80	0.41 ± 0.06	2.2 ± 0.4
100	0.55 ± 0.08	2.9 ± 0.7

Table 4: Shows superoxide anion scavenging activity of ethanolic fruit extract of *Calamus vattayila* and Ascorbic acid

Concentration of the extract (µg)	% inhibition of the extract	% inhibition of ascorbic acid
20	8 ± 0.04	49 ± 0.09
40	18 ± 0.09	56 ± 1.1
60	32 ± 0.4	68 ± 0.8
80	41 ± 0.6	82 ±1.3
100	65 ± 1.1	93 ±1.4

Table 5: Showing anti- arthritic activity of the ethanolic fruit extract of *Calamus vattayila* and Diclofenac sodium

Concentration of the extract and diclofenac sodium (µg)	% of inhibition of the extract	% of inhibition of diclofenac sodium
20	6 ± 0.01	55 ± 0.05
40	14 ± 0.03	71 ± 0.2
60	21 ± 0.06	85 ± 0.3
80	34 ± 0.08	88 ± 0.5
100	56 ± 0.1	94 ± 0.7

CONCLUSIONS

The results showed fruit extract of *Calamus vattayila* exhibited strong biological activity when compared with standards. The biological activity of the extract may be due to the presence of phenolic compounds. Protein denaturation is one of the main causes of rheumatoid arthritis due to the production of auto antigens. The results of the present study showed that the extract may control the production of auto antigens by preventing protein denaturation. The fruits of *C. vattayila* could be served as a new source of nutraceuticals with potential applications to reduce the level of oxidative stress and related health benefits.

ACKNOWLEDGEMENTS

The author is thankful to Prof. S.R. Ramesh, Chief Scientist, DOS in Life Sciences, Pooja Bhagavat Memorial Mahajana Education Centre, PG Wing of SBRR Mahajana First Grade College (Autononous), Mysuru.

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