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IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS ROOT AND RHIZOME EXTRACTS OF *ACORUS CALAMUS* LINN.

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ABSTRACT

Consecutive ethanolic and hydro alcoholic extracts of roots and rhizomes of *Acorus calamus* Linn (Araceae) were prepared and investigated for antioxidant potential against 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radicals and compared with butylatedhydroxyanisole (BHA) and silymarin. Cumulative concentrations of both the extracts showed free radical scavenging activity of 59.13 ± 18.95 and 56.71 ± 19.54 respectively. It was also concluded that ethanolic extract showed the maximum antioxidant activity. Also increase in concentration of the extract showed enhance in free radical scavenging activity.

KEY WORDS

Acorus calamus Linn, Antioxidant activity, DPPH

INTRODUCTION

Acorus calamus Linn. (fam: Araceae) is a semi-aquatic, perennial, aromatic herb with creeping rhizomes originating in Asia[1] but now widely distributed in Europe, North America, Africa, etc. The rhizome, root and leaf yield a light brown to brownish yellow volatile aromatic oil known as calamus oil. The yield of oil from different part of the plant is upto 1.8% in fresh rhizome, 1.5 to 3.5% in dried rhizome, about 0.2% in leaves and 0.12%in fresh aerial parts[2]. Bach (Hindi name of *Acorus calamus*) is an ingredient of several Ayurvedic recipes, which are used for the treatment of rheumatoid arthritis with pain, swelling and functional disability[3-5] and low-grade mentally retarded children[6-7]. Clinical trials of herbal preparations had also been carried out for schizophrenia containing brahmi, bachi and jyotismati[8]. The present study is intended to investigate the phytochemical, antibacterial and antifungal activity of various extracts obtained from the powdered roots and rhizomes of the plant. The present study revealed the first time of investigating the free radical scavenging activity of the roots and rhizomes part of Indian *Acorus calamus*.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh plant materials of *Acorus calamus* which were collected from Krishna delta region of Vijayawada City surroundings of Krishna district in Andhra Pradesh state, was authenticated by Dr.S.M.Khasim, Assistant Professor, Department of Botany, Acharya Nagarjuna University in Guntur. One of the plant specimens had been planted in KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada and a voucher (No: KVSR/PCRL/No: 0051/BN) had been deposited after planted in the college herbal Garden.

Butylatedhydroxyanisole (BHA) and 2,2 diphenyl-1-picrylhydrazyl (DPPH) were purchased from B.D.H chemicals (P) Limited and S.D.Fine chemicals (P) Limited Mumbai. Silymarin was purchased from Sigma Aldrich, Mumbai. Other chemicals and reagents were procured from chemical laboratory of KVSR Siddhartha college of Pharmaceutical sciences, Vijayawada.

Extraction and Preliminary Phytochemical Screening

About 200 grams of the roots and rhizomes were subjected to hot soxhlete extraction[9] apparatus with 700ml of pet ether (60-80ºC) for 48 hrs. Then followed by 650 ml of ethanol (90%V/V) for another 48 hrs and the dried marc was subjected to cold maceration[10] by using 600mlof hydroalcohol (1:1) for 72 hrs. Finally the obtained extracts were filtered through muslin cloth. Then they were concentrated under reduced pressure and dried in vacuum condition to get a semisolid consistency whose yields are tabulated. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents[11-15] present in them.

Experimental work[16]

The free radical scavenging activity of the *A.calamus* root and rhizomes extracts were measured and compared with the activity of Butylatedhydroxyanisole (BHA) and silymarin for radical-scavenging potency using the stable radical 2,2 diphenyl-1-picrylhydrazyl (DPPH). The free radical scavenging activities of extracts, BHA and silymarin were measured by decrease in the absorbance of methanol solution of DPPH at 523 nm.

0.1mM solution of DPPH in spectrum alcohol was prepared and 1.5 ml of this solution was added to 3.5ml of extract solution, BHA and Silymarin in water at different concentrations (50-500mcg/ml). Thirty minutes later the absorbance was measured at 523nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability of the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = $[(A_{control} - A_{test}) / A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Statistical Analysis

All values are expressed as mean ±SEM. The data were statistically analysed by one-way ANOVA followed by Turkey kramper multiple comparison test. P values < 0.05 was considered as significant. were considered significant.

RESULTS AND DISCUSSIONS

DPPH (2,2 diphenyl-1-picrylhydrazyl) is a stable free radical system, which is employed as an essential model by *in vitro* antioxidant evaluation. In general in vitro methods are preliminary screening methods, which paves way for the *in vivo* evaluation.

In the present study, free radical scavengiong activity was carried on only two extracts which are polyphenolic enriched extracts. Ethanolic extract (59.13 ± 18.95) and hydro alcoholic extract (56.71 ± 19.54) are showing an excellent scavenging effect and significant to Silymarin(63.55 ± 17.88). But the synthetic drug butylatedhydroxyanisole (BHA) has more activity.

When an antioxidant is mixed with any concentration of the free radical forming sample

such as DPPH, it reduces the free radical formation, which is detected by decrease in the absorbance of DPPH presence at λ_{max} 523nm. Both the extracts are significant to the natural compound Silymarin as showed in Table 1. But the free radical scavenging activity of herbal standard was found to be less than the synthetic standard drug butylatedhydroxyanisole (BHA) at all dose levels. From this study it was concluded that free radical scavenging effect is tremendous for both the extracts at all the dose levels with that of the standard drugs.

Table1

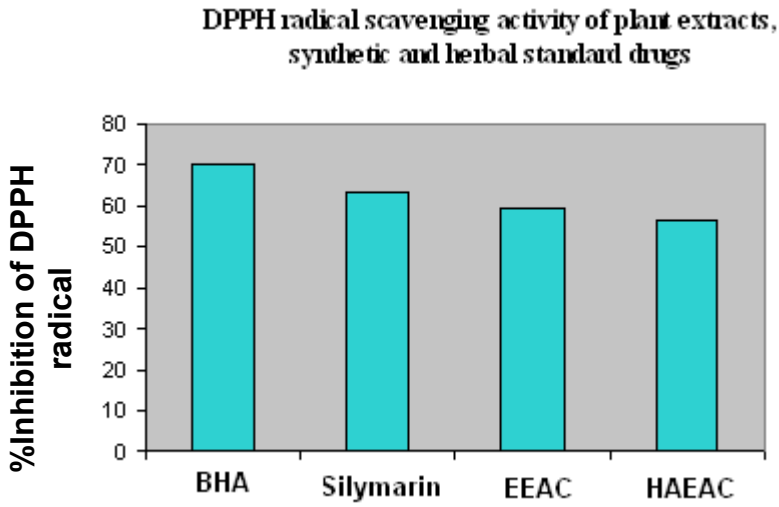
DPPH radical scavenging activity (antioxidant activities) of root extract of Acorus calamus Linn and other standard drugs

S.No	Compound	DPPH radical scavenging activity (% Inhibition ± SEM)
1.	Control	No activity
2.	BHA	70.37 ± 14.39
3.	Silymarin	63.55 ± 17.88
4.	EEAC	59.13 ± 18.95
5.	HAEAC	56.71 ± 19.54

BHA: Butylatedhydroxyanisole, EEAC: Ethanolic extract of *A.calamus* Linn.,
HAEAC: Hydro alcoholic extract of *A.calamus* Linn.

Figure 1

Bar Diagram Illustration of DPPH radical scavenging activity



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