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ANALYSIS OF ANTIOXIDANT IN CURCUMA ANGUSTIFOLIA RHIZOME BY 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) METHOD.

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ABSTRACT

Curcuma angustifolia also known as '*haledo'* is commonly found in Sikkim and has many medicinal uses and has been used traditionally since ancient ages. Its rhizome is aromatic and the solvent extract of the rhizome has free radical scavenging activity which was carried out in this study by 2,2-diphenyl-1-picrylhydrazyl DPPH. Hot methanol extract and cold methanol extract of the dried powder of the rhizome of *C.angustifolia* was used as sample for antioxidant profiling which was compared with two standards namely BHT (Butylated Hydroxyl Toluene) and Ascorbic acid. Further the percentage scavenging activity and the IC_{50} value was calculated.

KEYWORDS: ***C.angustifolia, Rhizome, Methanol, DPPH, antioxidant***

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INTRODUCTION

Curcuma angustifolia Roxb. under the family Zingerberaceae belongs to the genus *Curcuma* comprises of 80 species, (Sasikumar *et al;* 2005)[19] which also contain plants such as ginger and turmeric. This species have been gradually increasing in popularity in the Western hemisphere for its medicinal value, it is familiar to the Eastern hemisphere where it plays an important role in many Eastern cultures. (Ravindram et *al;* 2007)[18]. It has great medicinal value like anti-inflammatory (Yoshioka et *al;* 1998)[20], (Jain S.K. 1995)[8] and antimicrobial properties and is widely used in traditional medicine. Bioactive components are responsible for antioxidative and anti-inflammatory properties, wound-healing, anticoagulant, antimicrobial activities and (Chottopadhyay 2004)[5], antibacterial activities. (Rajeevkumar *et al;* 2010)[16]. It has also been reported to have analgesic effect. (Ali *et al;* 2004)[1], anti-allergic (Matsuda *et al;* 2004)[12], antioxidant (Mau *et al;* 2003)[13] and hepato-protective properties (Kim *et al;* 20005)[9]. The species of this plant is of great nutritional value, especially as a source of starch for Indian foods and medicines. The rhizomes of *Curcuma angustifolia* are typically grounds into flour, which can be mixed together with the milk or water to form a nutritious meal. (Ravindram *et al;* 2007)[18]. Most importantly it is used as an ingredient for the replacement of breast-milk, or as nutritional supplements for babies a short while after weaning. (Doble *et al;* 2011[6]. It can be used to heal peptic ulcers, is beneficial in treatment of dysentery. It is used as agreeable, non-irritating diet in certain chronic diseases as herbal tonic for patients suffering from tuberculosis used to sooth cough and is used to treat bronchitis. (Doble *et al;* 2011)[6]. Convalescence in fever, in irritation of alimentary canal, pulmonary organs and also used in consumption, excessive thirst, jaundice and kidney disorder. (Rao *et al;* 1914)[17]. Rhizomes and the leaves of *C.angustifolia* have a camphoraceous aroma and contain many functional compounds such as phenolics, flavonoids and different antioxidant enzymes. The importance of phenolic compounds in plants as natural antioxidant their use as substitutes to synthetic antioxidants in food additives is well known. (Branen 1975)[3], (Martinez 2001).[11]

MATERIALS AND METHODS

i. Collection of Plant material

The fresh rhizome of the *Curcuma angustifolia* was collected from Sajong Rumtek, East Sikkim in the month of December-January. It was then washed for several times to make it free of dust and mud particles, cleaned and weighed and was cut into small uniform pieces and finally shade dried till 70.4 % moisture content was lost. The dried rhizome pieces were then grounded to fine powder which was further used for extraction.(Harborne,1998)[7]

ii. Solvent Extraction

a) Hot Extraction

22.95g of powdered sample was taken in a round bottom flask to which 100 ml methanol was added .Hot extraction was carried out using soxhlet apparatus for continuous 8 to 10 hours at 80^oC .After cooling to room temperature it was then filtered using No. 1 filter paper. The filtrate was evaporated in rotary evaporator Buchi, (Heating Bath B-491) at 30^oC till all the solvent was evaporated to give thick semi solid extract. It was further dried in hot air oven and was weighed.

b) Cold Extraction

22.95g of powdered sample was taken in a round bottom flask to which 100 ml methanol was added .Cold extraction was carried out by keeping the round bottom flask at room temperature for 24 hours. It was the filtered using No. 1 filter paper to give residue and filtrate. The filtrate was evaporated in the rotary evaporator of Buchi, (Heating Bath B-491) at 30^oC till all the solvent was evaporated to give thick semi solid extract. It was further dried in open air at room temperature till all the solvent was evaporated and the solid extract was weighed and stored for further studies. (Harborne,1998)[7]

Antioxidant assay using DPPH method

Sample Preparation

50 mg of both hot and cold solid extract of the rhizome of *C.angustifolia* was weighed in the

empty vials and dissolved in 50 ml of methanol respectively to give mg/ml concentration which was further used as sample for free radical scavenging activity by DPPH assay. Similarly standard solutions were prepared by dissolving BHT and Ascorbic acid in methanol to give 1mg/ml concentration respectively.

Free radical Scavenging activity

Free radical scavenging activity was carried out by the method followed by (Cheel *et al;* 2007)[4] with some modifications. 0.006 mM of DPPH solution was freshly prepared in methanol and stored in dark reagent bottle. Different concentration of hot and cold extract sample solution was prepared by adding100 µl,200 µl,300 µl,,400 µl,500 µl,600 µl,700 µl, and 800 µl in clean test tubes respectively using micropipettes .Further the methanol was added to make it up to 1 ml.2ml of freshly prepared DPPH solution was added to all the test tubes and shaken well .It was further incubated at dark for 30 minutes and the reading was taken at 517nm using Visible spectrophotometer of Coslab make(CLE30). Methanol was taken as blank and control was prepared using 1ml of methanol to which 2ml of DPPH was added the reading was taken. The prepared solution of BHT and Ascorbic acid was taken as the standard and same procedure was carried out .Methanol was taken as blank and 1 ml methanol plus 2ml DPPH was taken as control .The procedure was carried out in duplicates and mean was taken (n=2).Further the percentage scavenging activity was calculated using the formula given below and scatter plot graph was plotted using Microsoft excel 2007 and IC_{50} value which is the effective concentration of the sample that cause 50% of inhibition that is loss of DPPH activity was calculated using Graph pad prism software(version 6.04):-

$$\% \textbf{ scavenging} = 1 - \frac{(\text{A sample})}{(\text{A control})}$$

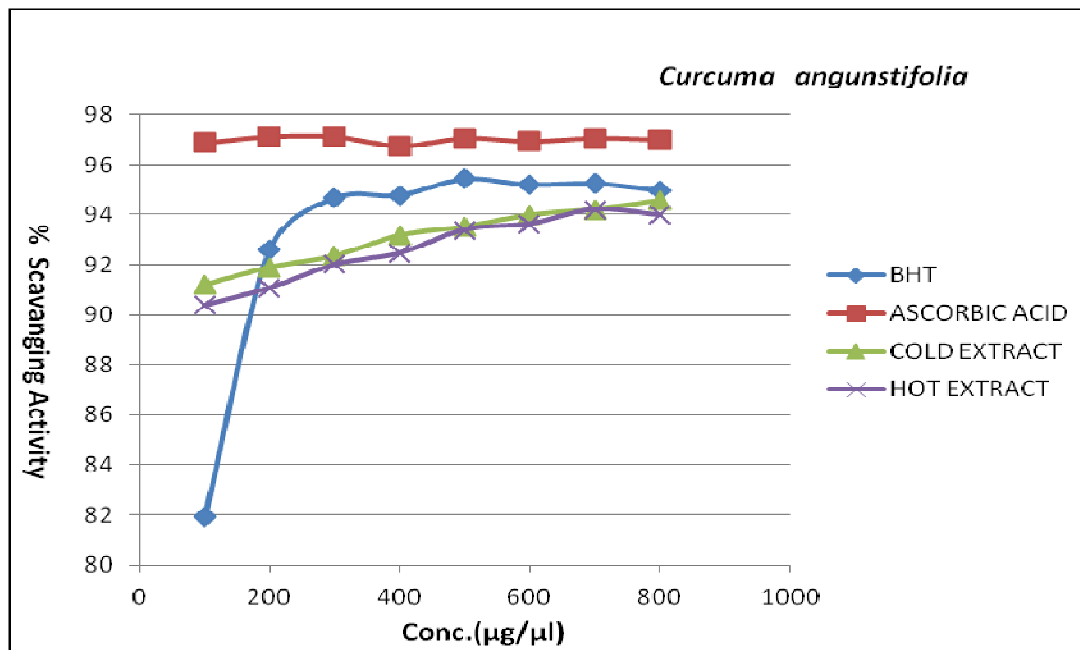

Figure-1

percentage scavenging activity of BHT (Butylated Hydroxyl Toluene) and Ascorbic acids as standard and cold and hot methanol extract of the rhizome of Curcuma angustifolia.

Table 1
IC50 value of cold methanol and hot methanol extract of Curcuma angustifolia rhizome (value calculated using Graphpad prism software.(6.04)

Sl. No.	SAMPLE	IC_{50} (µg/µl)
1	Cold Methanol Extract of *C.angustifolia*	357.4
2	Hot Methanol Extract of *C.angustifolia*	341.5

OBSERVATION & RESULT

Cold methanol extract and hot methanol extract of the rhizome of *Curcuma angustifolia* showed more or less similar antioxidant activity. Percentage inhibition of 100 µg/µl conc. of the cold extract which was the lowest concentration is 91.2 ± 0.02 and that of 800 µg/µl which was the highest concentration is 94.5 + 0.02.Similarly in case of the hot methanol extract of the rhizome of *C.angustifolia,* the percentage inhibition of the lowest concentration 100 µg/µl was 90.38 ± 0.01 and that of highest concentration 800 µg/µl 93.97 ± 0.02. Rhizome of *C.angustifolia* has less potential in free radical scavenging activity compared to the standards namely BHT and Ascorbic acid. Further the IC_{50} value for cold methanol extract and hot methanol extract of C.angustifolia was found to be 357.4 (µg/µl) and 341.5 (µg/µl) respectively.

DISCUSSION

Antioxidants are the compounds with free radicals scavenging activity and capable of protecting the cells from free radical mediated oxidative stress. The antioxidant compounds can be derived from natural sources such as plants. Antioxidant activity of these plants is due to the presence of flavones, isoflavones, flavonoids, carotenoids (Ali et al; 2004)[1], phenol (Bellary et al; 2012)[2], (Miquel et al; 2002)[14]. Free radicals are the cause of several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known (Paramapojn *et al;* 2004)[15], (Branen *et al*; 1975)[3]. Therefore, these observations could help in developing new drugs for the therapeutic use in human-beings. However, only limited work has been done on *Curcuma species* (Krishnaraj *et al* ; 2010)[10] .Therefore, the present work was aimed to analyse the antioxidant potential of this underutilised species of *Curcuma.*

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