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COMPARATIVE STUDY OF RADICAL SCAVENGING ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF FRESH AND DRY RHIZOMES OF *CURCUMA ZEDOARIA*

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ABSTRACT: Oxygen is an element obligatory for life. Biological combustion produces harmful intermediates called free radicals. Free radicals are continuously produced by the body's aerobic life and our metabolism. Antioxidants are the substances, which act against oxidative compounds. Under normal conditions the body's antioxidants convert ROS to prevent the over production of free radicals. Recently, natural foods and food derived antioxidants such as vitamins and phenolic phytochemicals, have received growing attention, because they are known to function as chemo preventive agents against oxidative damage and are considered beneficial for human health. The present study was conducted to compare the antioxidant activity of fresh and dry rhizomes of *Curcuma zedoaria.* The methanol extract of both the rhizomes showed good radical scavenging activity.

INTRODUCTION: Nature has provided a complete store house of remedies to cure all ailments of mankind [1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [2].

Majority of world's population depends on traditional medicine for primary health care. Plants have been extensively used as a rich source of medicine as they contain organic compounds with therapeutic value [3].

Recently there has been an increased interest in the food industry and in preventive medicine in the development of 'natural antioxidants' from plant material. Natural antioxidants are found in various vegetables such as carrot, beet, tomato, green tea leaves, soy bean, orange, malt grains and various spices such as cardamom, cinnamon, clove, coriander, ginger, garlic, etc [4].

Curcuma zedoaria is a rhizomatous species from the *Zingiberaceae* family commonly known as ginger family. "Ginger" is a general term for members or species of ginger families. *Curcuma zedoaria* is locally known as 'Kunyit putih' or 'temu putih' [5].

Curcuma zedoaria has been traditionally used in many countries especially in South East Asia as a valuable medicinal plant for many centuries to treat stomach diseases, blood stagnation, diarrhea, coryza, skin disorders, rheumatism and also used as hepato protective and for promoting menstruation [6].

In traditional medicine, the tubers of *Curcuma zedoaria* have been used as a carminative, digestive stimulant and for treatment of colds and infections. They also exhibit antibacterial and antifungal activities. Curcumin, dimethoxycurcumin and bisdemethoxy-curcumin isolated from this plant were reported for antioxidant and anti inflammatory activities [7].

The present study aims to compare the free radical scavenging activity of fresh and dry rhizomes of *Curcuma zedoaria* and to qualitatively determine the phytochemical constituents in the rhizome.

MATERIALS AND METHOD:

Plant material: The fresh and dried rhizome parts of the plant materials *Curcuma zedoaria* were collected. Fresh rhizome was collected by uprooting the plants growing in our University campus and dry rhizome was procured from local markets.

Preparation of Extract: About 5g of the fresh and dried powdered plant material were extracted serially with solvents in soxhlet extractor. The solvents used were petroleum ether, chloroform, ethyl acetate, methanol and ethanol. Final residue was extracted with water. Each extract was concentrated by distilling off the solvent and then evaporated to dryness. The extracts obtained were dissolved in Dimethyl sulphoxide (20mg/ 5µl) and subjected to qualitative test for the identification of various phytoconstituents and free radical scavenging activity.

Diphenyl picryl Hydrazyl (DPPH) radical scavenging activity

The ability of rhizome extracts to scavenge the stable free radical DPPH and convert it into Diphenyl picryl hydrazine was determined by the method described by Mensor *et al*., 2001 [8]. The scavenging ability of the extract was calculated by;

DPPH Scavenging activity (%) =

$$[(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample (i.e. extract).

Azino bisethylbezthiozoline sulphonic acid (ABTS) Scavenging Activity: The percent inhibitions of ABTS radical by rhizome extracts were determined as per Shirwaiker *et al.,* 2006 [9] and the percentage inhibition of the extract was calculated from the following equation:

% inhibition =

$$[(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

Hydrogen Peroxide Scavenging Activity: The ability of the rhizome extracts to scavenge hydrogen peroxide radical was determined by measuring the decrease in absorbance at 230nm spectrophotometrically, explained by Ruch *et al*., 1989 [10]. The percentage of H_2O_2 scavenged by the extract was calculated by the formula

Scavenging activity (%) =

$$[(\text{Abs control} - \text{Abs sample})] / (\text{Abs control})] \times 100$$

Hydroxyl Scavenging Activity: The extent of hydroxyl radical scavenging activity by rhizome extracts were measured spectrophotometrically by method described by Elizabeth and Rao, 1990 [11]. The assay quantifies the 2- deoxyribose degradation product, by its condensation with TBA.

Scavenging activity (%) =

$$[A_0 - A_1] / A_0 \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the samples.

Inhibition of Nitric oxide Generation: The extent of nitric oxide generation was studied using Griess reagent method explained by Green *et al.,* 1982 [12]. % inhibition of NO = Abs control / Abs sample × 100

Inhibition of Superoxide Generation: The extent of superoxide generation was studied by method described by Winterbourn *et al*., 1975 [13]. The difference in the optical density before and after illumination is the generation of superoxide by the extract was calculated by comparing with the optical density of the control

% inhibition of SO =

$$[\text{Abs control} - \text{Abs sample}] / \text{Abs sample} \times 100$$

Qualitative analysis of the Phytochemicals: The extract prepared was tested for the presence of alkaloids, flavonoids, phenolics, saponins, glycosides, steroids and terpenoids as per Khandelwal, 2002 [14] .

RESULTS AND DISCUSSION: When the extracts of fresh and dry rhizomes were assessed for DPPH scavenging acitivity as shown in **figure 1** the methanolic extract of fresh and dry rhizome exhibited strong DPPH radical scavenging activity and significantly higher compared to standard trolox. The petroleum ether, benzene, chloroform, ethyl acetate extracts of fresh rhizome showed higher ABTS scavenging activity when compared to the extracts of dry rhizome as shown in **figure 2**.

The methanol and ethanol extracts of dry rhizome exhibited stronger activity. When compared to standard, methanol, ethanol, ethyl acetate, chloroform, benzene extracts showed considerable activity while petroleum ether and aqueous extracts showed least activity. All the extracts except chloroform extract of dry rhizome showed significant H_2O_2 scavenging activity. The methanol extract of fresh rhizome showed significantly higher activity. When compared to standard, ethanol, ethyl acetate extract of dry rhizome showed similar activity and methanol extract of fresh and dry rhizome showed significant activity while chloroform, benzene, petroleum ether and aqueous extracts showed moderate activity. The results are presented **figure 3**.

For the assessment of hydroxyl scavenging activity H_2O_2 treated control was fixed to 100% and the value of other groups were compared relative to this. The percent of TBARS formed was compared. The aqueous extract of fresh and dry rhizomes inhibited TBARS to a significant extent compared to other extracts. When treated with H_2O_2 the % TBARS increased which was significantly lowered on treatment with both fresh and dry rhizome extracts. The activity of methanol and ethanol extracts of both the rhizomes was significantly higher compared to standard trolox.

The methanol extract of fresh and dry rhizome of *Curcuma zedoaria* decreased the generation of NO and SO significantly *in vitro*. When fresh and dry rhizome extracts were compared all the extracts expect ethanol extract of fresh rhizome showed higher activity. The scavenging effect of methanol extract of fresh rhizome was significantly more pronounced than standard trolox. In case of dry rhizome, its activity was comparable to the standard.

Antioxidant activity of *Curcuma zedoaria*:

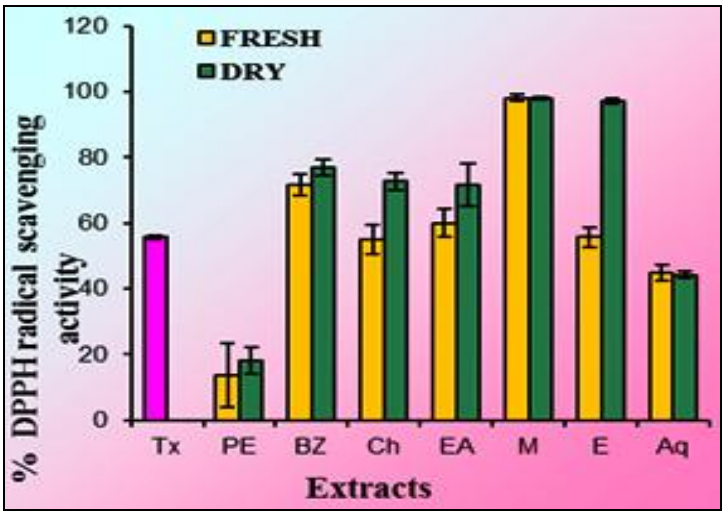

FIGURE 1: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF CURCUMA ZEDOARIA

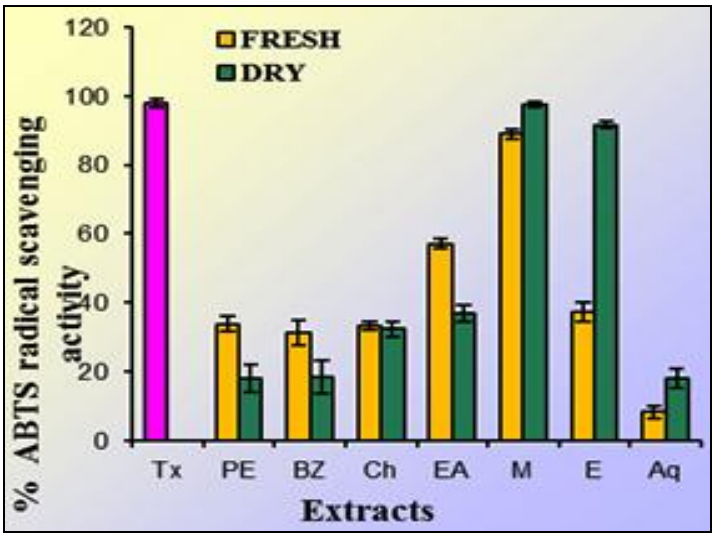

FIGURE 2: ABTS RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF CURCUMA ZEDOARIA

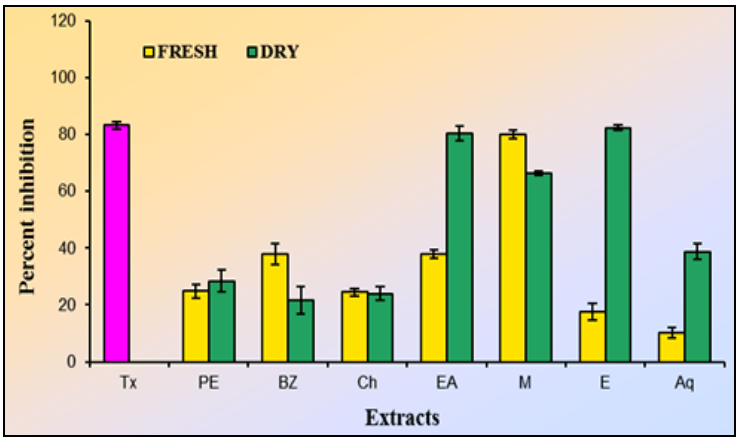

FIGURE 3: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF *CURCUMA ZEDOARIA*

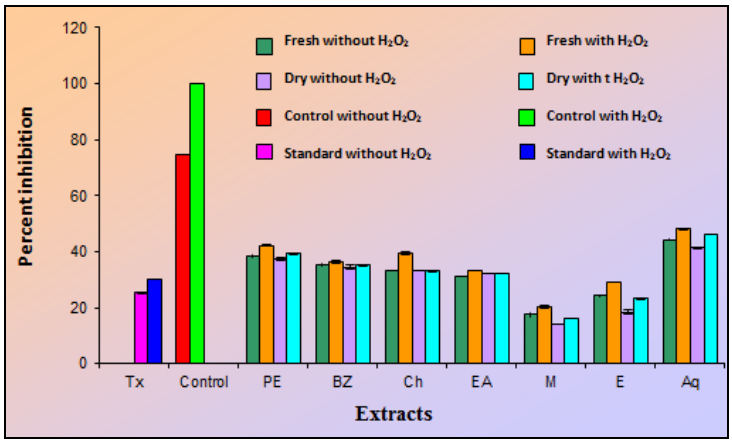

FIGURE 4: HYDROXYL RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF CURCUMA ZEDOARIA

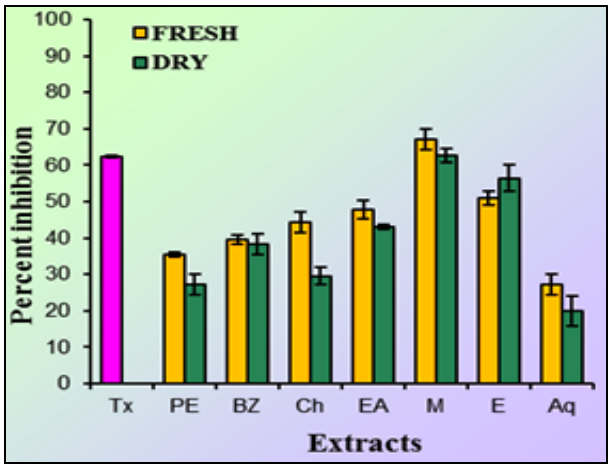

FIGURE 5: DETERMINATION OF INHIBITION OF NITRIC OXIDE OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF *CURCUMA ZEDOARIA*

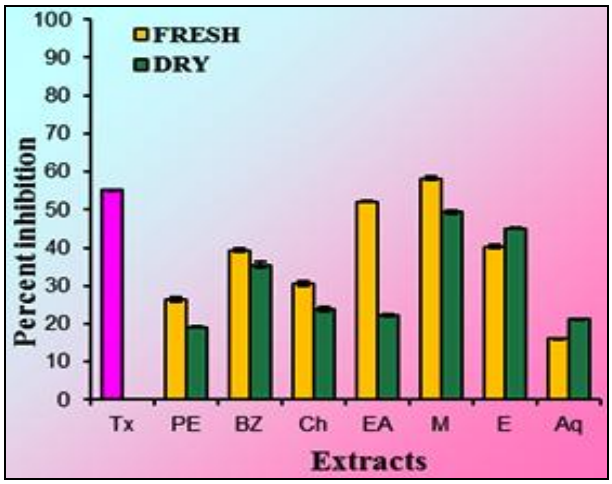

FIGURE 6: DETERMINATION OF INHIBITION OF SUPEROXIDE OF DIFFERENT EXTRACTS OF FRESH AND DRY RHIZOMES OF *CURCUMA ZEDOARIA*

Tx – Trolox; PE – Petroleum ether; BZ – Benzene; Ch – Chloroform; EA – Ethyl acetate; M – Methanol; E – Ethanol; Aq – Extracts

Phytochemical analysis of fresh and dry rhizomes of *Curcuma zedoaria*: Among all the extracts subjected to free radical scavenging activity, methanolic extract of fresh and dry rhizomes of *Curcuma zedoaria* showed higher antiradical activity. So methanol extract was used to determine phytochemical constituents. The extracts were subjected to qualitative analysis for detection of various plant constituents viz; alkaloids, phenolics compounds, flavonoids, saponins, glycosides, steroids, terpenoids and tannins. The results are depicted in the **Table 1.** In the methanol extract of fresh and dry rhizome of *Curcuma zedoaria* alkaloids, phenolics, flavonoids, saponins, glycosides, steroids, terpenoids were present and tannin was found to be absent.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF RHIZOMES OF *Curcuma zedoaria*

Phytochemical constituents	Fresh rhizome	Dry rhizome
Alkaloid	+	+
Phenolics	+	+
Flavonoids	+	+
Saponins	+	+
Glycosides	+	+
Steroids	+	+
Terpenoids	+	+
Tannins	-	-

+ - presence of compound; - - absence of compound

CONCLUSION: From the above results, the methanol extract of fresh and dry rhizome of *Curcuma zedoaria* showed similar activity. So these extracts can be exploited as future for medicinal use.

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