INTRODUCTION

Herbal products encompass a variety of self-prescribed preparations of plant origin that may be generally categorized as food, dietary supplements, cosmetics and herbal medicinal products [1]. The genus *Zingiber* has about 85 species of aromatic herbs mostly distributed in East Asia and tropical Australia [2]. Plants belonging to Zingiberaceae (Ginger family) are known for a number of medicinal properties [3-5]. The term ‘Zingiber’ is derived from the Sanskrit word ‘shringavera’, owing to their ‘horn-shaped’ rhizomes.

*Z. officinale* is one of the best-studied Zingiber oils. The oil consists of a complex mixture of essential oils, including monoterpene and sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, and oxygenated diterpenes [3, 6]. The rhizome oil of *Z. officinale* has been the most studied Zingiber oils. The oil varied from the rhizome oils of *Z. zerumbet* and *Z. officinale*, the most studied Zingiber oils [3, 6]. The oil showed significant activities against the plant pathogen Fusarium oxysporum [9].

Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired nascent electron. Atoms of oxygen or nitrogen having central unpaired electron, are called reactive oxygen or nitrogen species [10-13]. Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA estimation of total flavonoid content was carried out in three different extracts: n-Hexane, Chloroform and Methanol.

Methods: Comparative Nitric oxide scavenging, DPPH scavenging and superoxide scavenging were carried out in all the three sequential extracts of *Zingiber nimmonii* so as to calculate the free radical scavenging power of the plant. Estimation of the total flavonoid content was performed in the three extracts using Aluminium chloride method.

Results: n-Hexane extract gave an IC₅₀ of 180 μg/ml, 270 μg/ml, 326 μg/ml respectively for nitric oxide, DPPH and superoxide scavenging assays. Activities were greater than the positive controls which proved the significance of n-hexane extract compared with chloroform and methanol. Hexane extract also gave the maximum percentage of flavonoid content (20% equivalent to that of Quercetin standard) when compared to Chloroform (18%).

Conclusion: All the antioxidant assays and flavonoid estimation studies revealed that hexane was the most promising and significant extract among hexane, chloroform and methanolic extracts. So the potential extract was chosen to be hexane and was further screened for various characterization studies.

Keywords: Zingiber nimmonii, Zingiberaceae, Nitric oxide scavenging, IC₅₀.
Comparative graph was plotted so as to compare the potential of the immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. A Quercetin from a calibration curve. The calibration curve was spectrophotometrically. Extracts prepared in Hexane, Chloroform and Methanol, were added to different test tubes in varying concentrations (0-600 mg/ml). Sodium nitroprusside (5 mM) in phosphate buffer was added to each test tube to make volume up to 1.5 ml. Solutions were incubated at 25 °C for 30 minutes. Thereafter, 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. A Comparative graph was plotted so as to compare the potential of the n-Hexane, Chloroform and Methanolic extracts.

Nitric Oxide Scavenging Assay [20]
Nitric oxide scavenging activity was measured spectrophotometrically. Extracts prepared in Hexane, Chloroform and Methanol, were added to different test tubes in varying concentrations (0-600 mg/ml). Sodium nitroprusside (5 mM) in phosphate buffer was added to each test tube to make volume up to 1.5 ml. Solutions were incubated at 25 °C for 30 minutes. Thereafter, 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. A Comparative graph was plotted so as to compare the potential of the n-Hexane, Chloroform and Methanolic extracts.

DPPH Radical scavenging Assay [21]
Various concentrations of plant extracts (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.1 mM. The mixture were shaken vigorously and left to stand for 30 min and the absorbance was measured at 517 nm. BHT was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

\[ \text{% decolorization} = \left[ 1 - \frac{\text{ABS sample}}{\text{ABS control}} \right] \times 100 \]

Superoxide Radical Activity [22]
This method is based on the inhibition of production of nitroblue tetrazolium of the superoxide ion by the plant extract and is measured spectrophotometrically. The assay tubes containing 20 µl of methanolic extract, 0.2 ml of EDTA, 0.1 ml of nitroblue tetrazolium, 0.05 ml of riboflavin and 2.25 ml of phosphate buffer and control tubes were set up without the extract. Similarly the activity of the standard antioxidants was also carried out. The initial optical uniformly with a fluorescent lamp for 30 min. A 560 nm was measured again and difference in optical density was taken as the quantum of superoxide production. The percentage inhibition was calculated by comparing with the optical density of the control tubes.

\[ \% \text{ scavenging of Superoxide} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100 \]

Determination of total flavonoid content
Colorimetric aluminum chloride method was used for flavonoid determination [23, 24, 25]. Briefly, 0.05 ml solution of each plant extracts were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Thermo Scientific U-2000 UV/Visible spectrophotometer. Total flavonoid contents were calculated as Quercetin from a calibration curve. The calibration curve was prepared by preparing Quercetin solutions at concentrations 20 to 100 µg/ml in methanol.

RESULTS AND DISCUSSION
Nitric Oxide Scavenging Assay
It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis [26]. IC50 values were calculated for each of the extracts and compared with Ascorbic acid standard. Hexane extract gave the most promising results. IC50 values were 180 mg/ml-1, 200 mg/ml-1, 301 mg/ml-1 for hexane, chloroform and methanol respectively as compared to the 190 mg/ml-1 of the ascorbic acid standard. The IC50 values of the hexane and chloroform extracts showed significant activity than A.purpurata of the same family.
were calculated for each of the extracts and compared with Ascorbic acid standard. Hexane extract gave the most promising results. IC₅₀ values were 3.26μg/ml, 3.36μg/ml, 2.25μg/ml for hexane, chloroform and methanol respectively as compared to the 40μg/ml of the Ascorbic acid standard [Fig 3]. The IC₅₀ values of the hexane and chloroform extracts showed significant activity similar to Ascorbic acid.

REFERENCES


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