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STUDIES ON IN VITRO FREE RADICAL SCAVENGING POTENTIAL OF ISOLATED AND PURIFIED COMPOUNDS FROM *CHRYSOPOGON ZIZANIOIDES* CHLOROFORM EXTRACT

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ABSTRACT To investigate the fractions of the chloroform extract of *Chrysopogon zizanioides L* by column and thin layer chromatography and antioxidant activity of fractions of chloroform extract of *Chrysopogon zizanioides L.* The extract was screened for possible antioxidant activities by free radical scavenging activity (DPPH) method. The results showed that all the plant fractions possessed antioxidant properties including radical scavenging activities. The anti-oxidative activities of four compounds were compared with the standard ascorbic acid. The compound four of *Chrysopogon zizanioides L* was more effective than that of other three compounds. This study suggests that the four compounds from *Chrysopogon zizanioides* L chloroform extract exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

KEYWORDS : *Chrysopogon zizanioides*, Column chromatography, Thin layer chromatography, Antioxidants.

INTRODUCTION

Antioxidants are substances that inhibit the oxidation process and act as protective agents. They protect the body from the damaging effects of free radicals (by-products of the body's normal chemical processes). Free radicals can attack healthy cells, thereby changing their DNA, which in turn causes tumors to grow. Research is currently underway to study the role of antioxidants in reducing the risk of cancer [1]. Free radicals or highly reactive oxygen species are formed by exogenous chemical substances or endogenous metabolic processes in the human body. They can oxidize biological molecules through nucleic acids, proteins, lipids and DNA, and can cause different diseases such as cancer. Antioxidants are compounds that stop the following molecules from attacking free radicals, thereby reducing the risk of these diseases [2]. With the help of enzymes such as superoxide dismutase, catalase, and antioxidants, almost all living things are protected to some degree from damage by free radicals [3].

Antioxidant supplements or dietary antioxidants can prevent the damaging effects of free radicals. Currently, a lot of attention is focused on the use of natural antioxidants to protect the human body, especially brain tissue, from oxidative damage caused by free radicals. In the past two decades, several medicinal plants have shown this effect through traditional cancer treatment methods [4]. Compared with existing medicines, traditional medicines based on plant extracts have been shown to be clinically effective and have relatively low toxicity. Free radicals released due to typical biochemical reactions in the body are related to cancer, ischemic heart disease, inflammation, diabetes, aging, atherosclerosis, immunosuppression and neurodegenerative diseases [5]. The human body has a characteristic barrier system that can fight free radicals (such as proteins), such as catalase, superoxide dismutase and glutathione peroxidase. Selenium, vitamin C, -carotene, vitamin E, lycopene, lutein and various carotenoids have been used as supplementary antioxidants. Therefore, flavonoids and terpenoids, the secondary metabolites of plants, play an important role in the defence of free radicals [6].

MATERIALAND METHODS

Sample collection

The medicinal plant used in the experiment is an aerial part of *Chrysopogon zizanioides L* were collected from a local medicinal farm. The plant materials were identified by reputed botanist from Madras Christian College, Tambaram, Chennai, India.

Preparation of extract

1,000 grams of plant material were placed in three separate round-bottom flasks to extract samples using solvents (chloroform). Extraction was performed with 250 ml of solvent mixture for 24 hours. At the end of the extraction, the respective solvent were concentrated under reduced pressure and placed in a water bath (50°C). Now store the extracted experimental solution in the refrigerator.

Chemicals and reagents

All chemicals used in this project were purchased from Sigma Chemical Company. USA.

Silica gel Column Chromatography

The chloroform crude extract was subjected to column chromatography fractionation technique. The column was prepared with silica gel (100- 200 mesh) with chloroform. About 5g of crude extracts was mixed with 10g of silica gel (1:2) separately (60-120 mesh[slurry]) and loaded in the packed column and eluted with 100% chloroform and followed by increasing the polarity by increasing chloroform: methanol starting at 100:0; 95:5; 90:10; 85:15;…0:100%. The column flow rate was adjusted to 1ml/min approximately 25 ml of 150 fractions was collected. TLC was carried out simultaneously for each fraction with the suitable mobile phase. The spots were visualized either by exposing to iodine vapours and/or UV light. The fractions showing the same R*f* was pooled together. Based on TLC profile 6 major fractions was separated, saved and kept under air current to facilitate drying. Further studies focused on antioxidant activity against DPPH free radical scavenging ability followed by anticancer property evaluation of major fractions which showed highest antioxidant assay and to identify active fractions using IR, MASS, NMR.

Thin layer chromatography

Thin-layer chromatography (TLC) is the simplest and cheapest method of detecting plant constituents since the method is easy to run, reproducible and requires little equipment [7]. TLC is an important method for the isolation, purification and confirmation of natural products. Compared with other chromatographic methods, TLC is often considered to be deficient in reproducibility and accuracy, but some distinctive attributes of this tool should be considered low-cost analysis, high-throughput screening of samples, minimal sample preparation, whole sample integrity, disposable stationary phase. Thin Layer Chromatography (TLC) is a solid-liquid type in which the two phases are a solid (stationary phase) and a liquid (moving phase). Solids most commonly used in chromatography are silica gel (SiO2 x H2O) and alumina (AL2O3xH2O). In our experiments thin layer chromatography (usually 5 µl of fraction) is loaded on pre-coated Merck TLC F254 TLC plates using chloroform: methanol (8:2) mixture as eluents.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

Antioxidant Assay – DPPH Method

Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The effect of given samples on DPPH radical was estimated according to the procedure described by [8]. Two mL of 6×10^{-5} M methanolic solution of DPPH were added to 50 µl of pooled compound dissolved in methanol. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows:

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of [9].

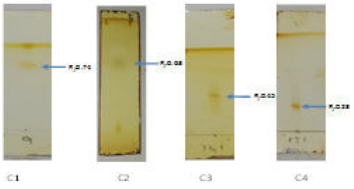
$IP = [(AC(0) - AA(t)/AC(0))] \times 100$

Where AC (0) is the absorbance of the control at t = 0 min; and AA (t) is the absorbance of the antioxidants at t = 16 min.

RESULTS AND DISCUSSION

Figure 1: Silica Gel Column chromatography of Chloroform extract

The result of column chromatography the crude sample gave 150 fractions which were eluted (Figure 1). TLC was carried out for each fraction with suitable mobile phase (chloroform: methanol (9:1)). The spots were visualized by exposing to iodine vapour and white light (Figure 2). Based on single spot by TLC profile, the fractions were pooled and combine to give 4 major fractions. In column chromatography, all the 4 fractions showed single spot-on TLC and Rf values were calculated (Table 1). All the 4 major fractions were subjected antioxidant potential analysis using DPPH free radical scavenging ability.



TLC Chromatogram developed on Methanol /chloroform (9:1) and Visualized under Iodine vapor

Figure 2: TLC Profile of purified compounds

Qualitative methods using Chromatographic Fingerprints are widely used to identify plant species due to their ability to provide a large amount of chemical information from complex matrices. Therefore, fingerprints play an important role in the characterization/ identification of chemical markers and other unknown compounds present in complex matrices. The identification of chromatographic regions and the assessment of similarities and differences are essential for the characterization of typical chemical characteristics, which is essential for performing quality control and correlating chemical content with species biological activity. TLC bands showed that the analytical performance of the chemical markers has important similarities through the two chromatographic techniques. Thus, simplifying the use of the developed Fingerprint operation is an auxiliary tool used to identify or identify the medicine components, extracts and fractions in chloroform extract of *Chrysopogon zizanioides* [10].

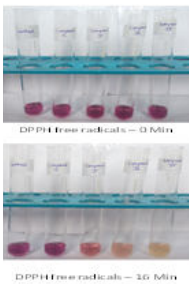


Figure 3: DPPH Radical Scavenging ability of purified compounds

Table 1: Different compounds and its Rf values

Compound	R_f value
C1	0.74
C2	0.63
C3	0.45
C4	0.23

Antioxidant activity of purified compounds showed different spectrum of the DPPH free radical scavenging ability. It was found that, the purified fractions showed different spectrum of spectrum of the DPPH free radical scavenging. The DPPH free radical scavenging ability is given in Table 2. The DPPH free radical scavenged by the purified compounds was showed after 16 min of incubation (Figure 3).

Table 2: Different compounds and antioxidant potential against DPPH free radicals

Compound	% DPPH free radical inhibition
Compound 1	27.07
Compound 2	43.15
Compound 3	54.78
Compound 4	80.10

The successful identification of biologically active compounds from plant materials mainly depends on the type of solvent used in the extraction process [11]. Many solvents have been used to extract active substances from plants, such as alcohol (ethanol or methanol), ether, chloroform, ethyl acetate, n-butanol and water [12]. Phytochemicals (secondary metabolites) are biologically active chemicals derived from plants. All parts of them in the plant are naturally synthesized: bark, leaves, stems, roots, flowers, fruits, seeds, etc. [13]. They are considered the basis of traditional herbal medicine practiced in the past and present [14]. All plant parts are usually screened for possible phytochemicals. The presence of phytochemicals of interest may lead to their further isolation, purification and characterization. It can then be used as the basis for new medicines.

The reactive oxygen species (ROS) formed in the body, such as superoxide anion (O2-), hydrogen peroxide (H2O2) and hydroxyl radicals (HO˙) are highly reactive chemical species that can be produced endogenously or internally or originated exogenously. Excessive production of ROS can lead to oxidative stress, leading to a variety of diseases. In this case, antioxidant compounds need to be taken in the diet to help the body neutralize free radicals to eliminate the harmful effects of oxidative stress. It is known that fruits, vegetables, grains and medicinal plants contain many phenolic compounds with strong antioxidant activity. It has been found that these compounds are closely related to the antioxidant capacity [15].

CONCLUSION

Natural antioxidants obtained from plant extracts or plant-derived isolated products have gradually replaced synthetic antioxidants with high safety. This study aims to determine the ROS scavenging inhibitory activity of medicinal plant *Chrysopogon zizanioides* and in order to evaluate the potential as a source of natural antioxidants. The present study provides the useful information about proximate composition, antioxidant properties of the chloroform extract of *Chrysopogon zizanioides*, which are used for the therapeutic purposes. The findings of this study support the fact that medicinal plants commonly consumed in India are promising sources of potential antioxidants.

REFERENCES

[1] Halliwell B, Gutteridge JM. (1984), "Oxygen toxicity, oxygen radicals, transition metals and diseases." Biochem J, 219:1–4.
[2] Maxwell SR. (1995), "Prospect for the use of antioxidant therapies." Drugs, 49: 45–361.
[3] Rice-Evans CA, Miller NJ, Paganga G. (1996), "Structure antioxidant activity relationships of flavonoids and phenolic acids." Free Radic Biol Med, 20:933–56.
[4] Prior RL, Cao G. (1999), "Variability in dietary antioxidant related natural product supplements: The need for methods of standardization." J Am Nutraceutical Assoc, 2:46–56.
[5] Awaad AS, El-Meligy RM, Qenawy SA, et.al. (2011), "Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants." Journal of Saudi Chemical Society, 15-4, :367–373.
[6] Lalitha TP, Jayanthi P. (2012), "Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms." Asian Journal of Plant Science & Research, 2:115–122.
[7] Marston A, Hostettmann K. (1985), "Plant molluscicides." Phytochem, 24(4): 639–652.
[8] Von Gadow A, Joubert E, Hansmann CF. (1997), "Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), x-tocopherol, BHT, and BHA." Journal of Agriculture and Food Chemistry, 45:632-638.
[9] Yen GC, Duh PD. (1994), "Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species." J Agric Food Chem, 42: 629–632.

[10] Gu M, Zhang G, Su Z, et.al. (2004), "Identification of major active constituents in the fingerprint of *Salvia miltiorrhiza* Bunge developed by high-speed counter-current chromatography." J. Chromatogr. A, 1041:239-243
[11] Poulson H, Preime S. (1998), "Loft Role of oxidative DNA damage in cancer initiation and promotion." European Journal of Cancer Preventive, 7:9–16.
[12] Govindarajan R, Vijayakumar M, Pushpangadan P. (2005), "Antioxidant approach to disease management and the role of Rasayana herbs of Ayurveda." Journal of Ethnopharmacology,99-2:165–178.
[13] Chanda S, Kaneria M, Nair R, (2011), "Antibacterial activity of Psoralea corylifolia L. seed and aerial parts with various extraction methods." Research Journal of Microbiology, 6- 2: 124–131.
[14] Adeshina GO, Onaolapo JA, Ehinmidu JO, and L. et.al. (2010), "Phytochemical and antimicrobial studies of the ethyl acetate extract of Alchornea cordifolia leaf found in Abuja, Nigeria." Journal of Medicinal Plants Research, 4-8:649–658.
[15] Grice HC. (1986), "Safety evaluation of butylatedhydroxytoluene (BHT) in the liver, lung and gastrointestinal tract." Food ChemToxicol, 24:1127-30.