IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2016 Vol.:5, Issue:3

Comparison of Different Extraction Methods of (*Zingiber officinale*) on Chemical Composition, Antioxidant Activity

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ISSN 2349-7203
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Submission: 10 January 2016
Accepted: 15 February 2016
Published: 25 February 2016

www.ijppr.humanjournals.com

Keywords: *Zingiber officinale*, extraction, DPPH assay, antioxidant activity

ABSTRACT

In this study, an evaluation of the chemical composition, antioxidant activity of the obtain extracts of Rhizome ginger *Zingiber officinale* by using different methods and solvents. Antioxidant activity on 2, 2-diphenyl -1-picrylhydrazyl (DPPH) radical was carried out. Ginger roots had been extracted by solvent extraction, maceration for sample(1, 2), continuous solvent extraction sample(3) and Super Critical Fluid Extraction, sample(4). The phytochemical analysis showed that the Ginger roots extractions, samples (1, 2, 3, 4) contain a number of medicinally important compounds in different amounts such as Tannins, Glycosides, Resins, Flavonoids, Alkaloid, Terpenes, Saponin. These various antioxidant activities of samples had been compared with ascorbic acid, butylated hydroxytoluene (BHT) as a standard antioxidant. The results showed that the ability of samples (3, 4) extractions to remove free radicals (Ec_{50}) less than (10mg/ml), this confirms to be more active as antioxidants comparing with samples (1, 2) extracts.

INTRODUCTION

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive. ROS, which included free radicals such as superoxide anion radicals ($O^{\cdot}_2$), hydroxyl radicals ($OH^{\cdot}$) and non free-radical species such as H_2O_2 and singlet oxygen ($^1O_2$), are various forms of activated oxygen[1]. *Zingiber officinale* Roscoe is a perennial aromatic plant belongs to the family *Zingiberaceae*, mostly distributed in East Asia and tropical Australia, the rhizomes of which are used as a spice [2]. This research has been performed to assess the antioxidant effect of the crude extracts of dried rhizome powder of *Zingiber officinale* plant which contains a number of antioxidant such as beta-carotene, ascorbic acid, terpenoids, alkaloids and polyphenols such as flavonoids, flavones glycosides, rutin, etc.[3]. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[4]. Several methods are used to measure the antioxidant activity of a biological material. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is the most common method that widely used due to their ease, speed and sensitivity[5]. DPPH is a dark-colored crystalline powder composed of stable free radical molecules. It is a stable radical because its spare electron delocalization over the whole molecule, gives the violet color to its solutions; when a hydrogen atom is donated to the molecule, a stable nonradical form of DPPH was formed changing its color to pale yellow[6].

The aim of this study is to evaluate the antioxidant activity of crude extracts of *Zingiber officinale*, using different methods and solvents.

FURTHER INFORMATION

Zingiber officinale Roscoe, collection:

The medicinal plant used in this study purchased from local herb store in Baghdad, classified by botany specialist.

Preparations of aqueous extract, sample(1):

250 ml of distilled water was added to 50 grams powder of *Zingiber officinale* and placed on the shaker for 24 hours at 37˚C. The extract was filtrated then was drying using Spray dryer at (45-50)˚C. The fine powder of the crude extract was weighed and stored in dark bottle at 4˚C.

Preparations of ethanolic extract, sample(2):

250 ml of 70% ethanol was added to 50 grams powder of *Zingiber officinale* and placed on the shaker for 24 hours at 37˚C. The solvent was eliminated under reduced pressure. A semi-solid extract was obtained after drying using Oven under vacuum at 40˚C. The crude extract was weighed and stored in dark bottle at 4˚C.

Extraction using continuous solvent extraction system, sample(3):

100 grams powder of *Zingiber officinale* was extracted with 1L of 80% ethanol at 60˚C for six hours in soxhlet apparatus. The extract was concentrated under reduced pressure using rotary evaporator. A semi-solid extract was obtained after drying, using Oven under vacuum at 40˚C. The crude extract was weighed and stored in dark bottle at 4˚C.

Extraction using Super Critical Fluid Extraction (SCFE), sample(4):

350 grams of dried *Zingiber officinale* was pulverized and sieved through 35/40 mesh (approximately 0.5mm diameter), prior to extraction with SC-CO_2 lab scale by Supercritical Fluid Technologies, Inc. US. (SFT-250 SFE system), using 250 bar at 40˚C, and was packed into a stainless steel column. CO_2 (>99.5% purity) was used as extraction solvent. The extract was taken and stored in dark bottle at 4˚C.

Phytochemical analysis:

Phytochemical analyses of the four crude extracts *Zingiber officinale* Roscoe that obtained by different methods[7], indicated different amount of tannins, carbohydrate, glycosides, resins, flavonoids, saponin, alkaloid and terpenes.

DPPH Radical Scavenging Activity:

Principle:-

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

$$(DPPH) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$

(Purple) (Yellow)

Antioxidants react with DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [8].

Butylated hydroxytoluene (BHT) and Vitamin C:

BHT and Vitamin C were artificial and natural antioxidant compounds used as positive control to evaluation the antioxidant activity of *Zingiber officinale* extractions. Concentrations (5, 10, 15, 25, 35 and 50 mg/ml) of BHT and vitamin C was prepared by dissolving (0.005, 0.01, 0.015, 0.025, 0.035 and 0.05 g), respectively by methanol for (BHT) and distilled water for (vitamin C). Then the volumes were completed to (1 ml)[9].

DPPH assay:

In order to obtain an indication of the antioxidant activity of *Zingiber officinale* extractions, 5 ml of a freshly prepared 0.004 % of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 µl of different concentration 5, 10, 15, 25, 35 and 50 mg/ml of each sample(1, 2, 3, 4) and the absorbance of each dilution after 30 minutes, was measured at 517 nm. Butylated hydroxytoluene (BHT) and vitamin C was the antioxidant used as positive control[10].

All tests were performed in triplicate and the methanol was used as blank solution. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

$$\% \text{ Reduction} = (\text{Abs DPPH} - \text{Abs Dil.})/\text{Abs DPPH} \times 100$$

Whereby:

Abs DPPH = average absorption of the DPPH solution

Abs Dil. = average absorption of the three absorption values of each dilution

With the obtained values, a graphic was made using Microsoft Excel. The Ec_{50} of each extract (concentration of extract or compound at which 50% of DPPH is reduced) was taken from the graphic.

Statistical analysis:

The statistical analyses were performed using the SPSS Ver. 19 program (Systat Software Inc., Chicago, IL, USA). Values were compared to control using analysis of variance (ANOVA) followed by Duncan's post hoc test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Table (1) shows the active compounds, results of phytochemical screening of *(Zingiber officinale*) extracts, samples (1, 2, 3, 4). This division base on composition of metabolic in cells and tissues of medical and aromatic plants which called natural products, that's a compound constitutes a group of secondary metabolites, quite widespread in nature with several therapeutical properties[11].

Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties[12]. In addition, phenolic compounds existing in plants are also responsible for their contribution to color, sensory and antioxidant properties of food[13]. Polyphenols consisting of a wide range of biogenic molecules play numerous roles in living organisms[14].

Table (1) Detection of active compounds of *Zingiber officinale* extracted with different methods and solvents.

Active compounds	Sample(1) pH=4.5	Sample(2) pH=5.5	Sample(3) pH=5.5	Sample(4) pH=5.5
Tannins	+	+	++	+
Glycosides	+	++	+	+
Resins	+	+	++	+
Flavonoids	+	+	+	+
Saponin	-	+	++	+
Terpenes	+	++	++	+
Steroids	-	-	-	-
Carbohydrate	+	+	++	+

Successful prediction of crude extracts from plant material are largely dependent on the type of solvent used in the extraction procedure, and the relative proportion between the amount of plant used for extraction and crude product was variable depending on several factors, such as method of extraction and solvent used in the extraction process as well as the type of study plant[15].

Table (2) shows the DPPH scavenging activity of *Zingiber officinale* extracts, samples (1, 2, 3, 4) at different concentrations, as assayed by DPPH (EC_{50} mg/ml) and BHT. Figure (1) illustrates the concentration of DPPH radical due to the scavenging ability of the extracts, samples (1, 2, 3, 4) for *Zingiber officinale* and standards. BHT and Vitamin C were used as references. Effectiveness of antioxidant properties is inversely correlated with EC_{50} values. If the EC_{50} value of an extract less than 10mg/ml, that's mean the extract is an effective antioxidant[16].

In this study, the EC_{50} value of sample (3) was 4mg/ml less than 10mg/ml also for sample (4), this indicates that the samples (3,4) were an effective antioxidant, Table (2).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[17]. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. The purple colored alcoholic solution of DPPH radical changes to yellow in the presence of hydrogen-donating antioxidant which could be measured at 517nm, the activity is expressed as effective concentration EC_{50}, which is the concentration of the sample leading to 50% reduction of the initial DPPH concentration[10].

Table (2): DPPH scavenging activity of *Zingiber officinale* extracts.

DPPH Conc.	Reduction % EC_{50}					
	Sample(1)	Sample(2)	Sample(3)	Sample(4)	Vit.C	BHT
5	23.27	17.08	74.27	61.40	87.60	85.96
10	30.17	28.07	75.67	72.28	90.52	87.01
15	30.67	34.27	80.35	77.19	91.35	87.13
25	31.11	43.74	81.05	82.22	91.23	87.25
35	32.63	50.76	85.03	83.27	89.00	87.95
50	45.38	58.01	87.25	87.49	87.95	87.95

The antioxidant activity significantly ($P < 0.05$).

(Conc.): Concentration, Vit.C: Vitamin C, BHT: Butylated hydroxytoluene.

P value was (0.02%) < (0.05%), so there is considered significant between the four different samples (1, 2, 3, 4). The (50%) concentration of each sample has the best result at the value (45.38, 58.01, 87.25, 87.49) respectively, for that the sample (4) had the best effect as antioxidant.

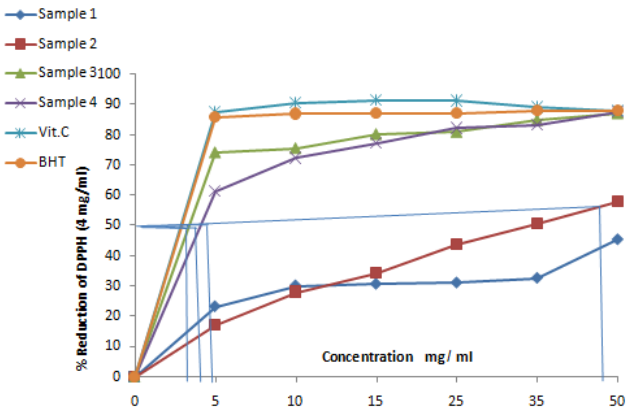

Figure (1): The reduction percentage of DPPH for different samples of *Zingiber officinale* extracts with references, natural source Vitamin C and artificial source BHT.

The FTIR Spectrum for the samples (2, 3, 4), using Infrared Spectroscopic Techniques (Tensor 27-PRUKER), shows in figures (2)(3)(4) respectively and the function groups were confirmed, Table (3) [18].

Table (3): The FTIR absorption for the function groups.

Samples No.	The absorption (cm^{-1})					
	C-H(St.)* Aliphatic	C-H(St.) Aromatic	O-H (St.)	C=C (St.)	C=O (St.)	C-O (St.)
2	2980	-	3346	-	1642	1085
3	2968, 2926	3010	3356	1602, 1516	1708	1085
4	2960, 2925, 2856	3021, 3078	3452	1515, 1450	-	-

*(St.): Stretch

Many authors discuss the chemical composition of *Zingiber officinale*, but no toxic effect was found[19].

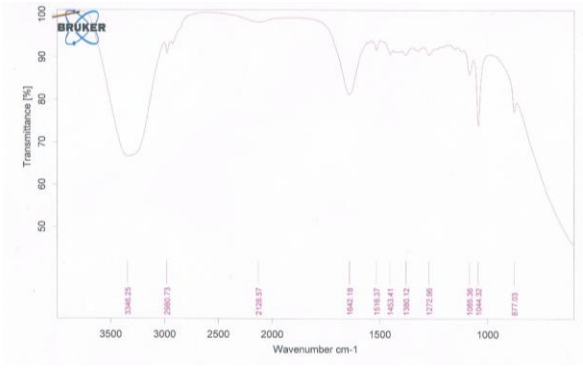

Figure (2): The FTIR Spectrum of the sample (2).

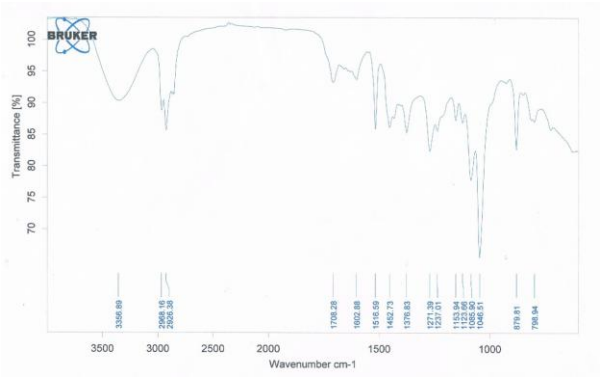

Figure (3): The FTIR Spectrum of the sample (3).

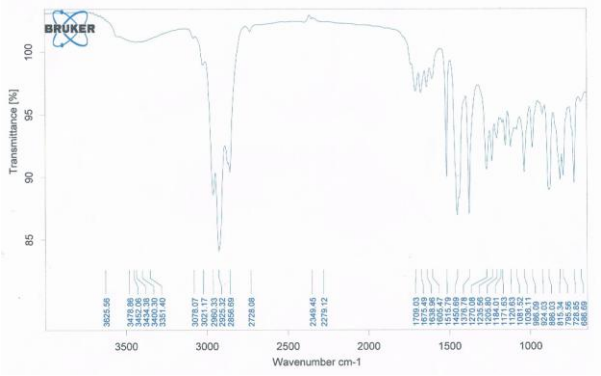

Figure (4): The FTIR Spectrum of the sample (4).

CONCLUSION

This study showed clearly the different effects of the extracts as antioxidant, confirmed with the positive control (BHT and Vitamin C). Samples (3, 4) more active as antioxidant than samples (1, 2), this effect can be attributed to the different solvent that used in extraction of *Zingiber officinale* with different methods.

ACKNOWLEDGMENT

The authors thanks Ibn Al-Betar Research Center, Corporation of Research and Industrial Development, Ministry of Industry and Minerals.

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