INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 4, p. 151-160, 2013

RESEARCH PAPER OPEN ACCESS

Screening of phytochemical compounds and antioxidant properties in local and HYV of Bangladeshi rice (*Oryza sativa* L.)

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Key words: Antioxidant activity, methanolic extract, screening, rice.

doi: http://dx.doi.org/10.12692/ijb/3.4.151-160 Article published on April 22, 2013

Abstract

Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells where consumption of whole grain plays a vital role. As a dietary supplement, antioxidant activities of five local and HYV rice (Kalijira, Chinigura, Hizoldigha, BRRI dhan28, BRRI dhan29) of Bangladesh were examined through DPPH antioxidant assay. Methanol extract of bran, polished and unpolished grain of each genotype were used as a studied sample. Studied sample showed significant antioxidant activity. Where bran is more potent part of rice showed higher antioxidant properties compeering unpolished and polished grain. Unpolished grain also showed greatest result where polished grain showed less performance. Among different genotypes Kalijira bran is black in color and showed better scavenging activity with the IC50 value of 60.12 µg/ml. Hizoldigha unpolished grain is red in color and showed higher antioxidant properties (130.2 µg/ml) compeering other unpolished grain. IC50 value of the positive control as BHT was 37.35 µg/ml. The result of present investigation denotes that the studied genotypes possess moderate antioxidant activity where Kalijira bran bear high antioxidant compound and keep demand to more processing and recently is using for extracting edible oil commonly called as rice bran oil. Unconventional Hizoldigha grain also contain high antioxidant activity and can be considered as nutraceutical foods as staple food.

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Introduction

It is widely recognized that dietary ingredients have a dual role, one of them is nutritional and another is pharmaceuticals. So now it's often called nutracuticals. In recent years, cereals and its ingredients are accepted as functional foods and nutraceuticals because of providing dietary fiber, proteins, energy, minerals, vitamins and antioxidants required for human health. Plant derived antioxidant such as ascorbic acid, tocopherols, carotenoids and phenolic compounds (polyphenols) (Choi *et al.,* 2007), besides other bioactive compounds are reported to have antioxidants activity. Currently, synthetic antioxidants such as butylated hydroxytoluene (BHT) butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are used under strict regulations because of their toxic effects on human enzyme systems (Hatate *et al.,* 1990, Hattori *et al.,* 1998). In contrast, natural antioxidants have attracted more and more interests because of their safety and wide distribution properties (Lewis, 1993).

The phytochemicals in fruits and vegetables are different from those in the grains, which contain tocotrienols and tocopherol, while rice is contain oryzanol (Lloyd *et al.,* 2000). The phenolic like ferulic acid and diferulate are predominant in grains, but are not significant in some fruit and vegetables (Bunzel *et al.,* 2001). Thus, the regular insertion of cereals and their processed products can make a payment to health endorsement and disease avoidance (Chaturvedi *et al.,* 2011).

Rice, being one of the most produced and consumed cereals in the world (FAO, 1995), has an important role in the relation between the diet and health. Several compounds with antioxidant activity have been identified in rice, including phenolic compounds, tocopherols, tocotrienols and γ-oryzanol (Iqbal *et al.,* 2005). Among them phenolic compounds is one of most important that are secondary metabolites of plants, with different activities such as protection against pathogens and predators, mechanical support, attraction of pollinating animals, and protection against ultraviolet radiation (Parr and Bolwell, 2000). Several phenolic compounds have already been identified in rice. The phenolic compounds are mainly associated with the pericarp in rice; hence, the milling process reduces the concentration of these compounds in the grain. Besides, grains with darker pericarp colour, such as red and black rice, contain higher amounts of polyphenols (Tian *et al.,* 2004). The concentration of total phenolics in the grain has been positively associated with the antioxidant activity (Zhang *et al.,* 2006).

Rice bran is an underutilized co-product from rice milling and generally used as animal feed, although it has long been considered an excellent source of vitamins and other nutrients. Bidlack (1999) has shown that rice bran may contain over 100 different antioxidants. Lloyd *et al.* (2000) also reported that, rice bran contains high amounts of beneficial antioxidants including tocopherols, tocotrienols, and oryzanols. It is also remarkable that, antioxidants containing level also depend on the type of rice (Gaydou *et al.,* 1980). However if we see the rank of antioxidant rich food, than it will be clearer that the color fruits, vegetables, spices and nuts are more potent to show antioxidant activity than grain. But all of those are expansible and not edible as much as we need where rice is only foods that we take maximum amount per day and suitable for all classes of people. So if we could find out the high antioxidant compound containing rice genotypes and increase the amount of those phytochemicals in our daily diet rice, than it would be also beneficial like *golden rice*. Studied genotypes Kalijira and Chinigura are local aromatic varieties and small in size, Hizoldigha is low yielding local *Amon* varieties with red color pericarp and normally grown in deep water where BRRI dhan28 and BRRI dhan29 are modern transplanted high yielding varieties of Bangladesh.

The present investigation was designed to evaluate the phytochemical screening and antioxidant activity

of rice genotypes generally cultivated if Bangladesh and are important in different aspects. Here DPPH antioxidant assay was used to evaluate the antioxidant activity of selected sample because scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay (Kordali *et al.,* 2005).

Materials and methods

Plant materials

Five Bangladeshi rice genotypes namely Kalijira, Chinigura, Hizoldigha, BRRI dhan28 and BRRI dhan29 (Fig. 1) were collect from farmers of Rajshahi region and the Regional Research Station of Bangladesh Rice Research Institute (BRRI), Rajshahi, Bangladesh and three part of each rice (Bran, polished and unpolished grain) were used as plant materials.

Fig. 1. Studied rice genotypes.

A= Kalijira, B= Chinigura, C= Hizoldigha, D= BRRI dhan28, E=BRRI dhan29

Preparation of extracts

The extraction procedure was done according to Ahmad and Beg (2001) with some modification. Collected rice were separated to bran, unpolished grain and polished grain by different milling process and made into fine powder. 50g fine powder was dipped into 250 ml methanol and left for 7 days with occasional shaking. Then Teton cloth and Whatman No. 1 filter paper was used for filtration. Filtrates were taken into glass beaker for evaporating solvent (methanol). For quick evaporating extra solvent from extract, water bath (4 holes analogue, Thermostatic water bath, China) was used under 60°C and stored at 4°C (Akueshi *et al.,* 2002). To calculate yield performance of the extract, standard formula was used according to Ekwenye and Elegalam (2005). Particular concentrations (31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, 650 µg/ml, 1,000 µg/ml; Note: 1 ppm = 1 mg/l) of the plant extracts were prepared.

Phytochemical analysis

Phytochemical analysis was carried out by Sofowara 1993, Trease and Evans 1989 and Harborne 1973, and the results observed were based on the colour change or precipitate formation after the addition of specific reagents.

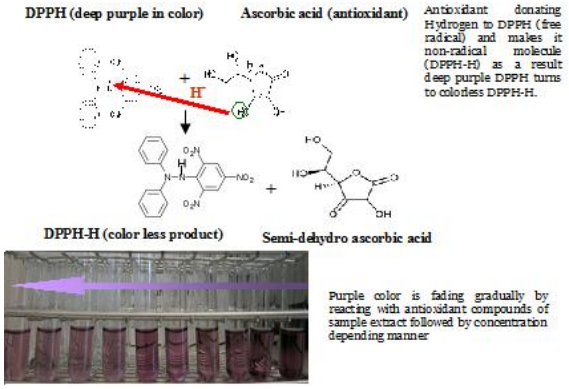


Fig. 2. Antioxidant donating Hydrogen to DPPH (free radical) and makes it non-radical molecule (DPPH-H) as a result deep purple DPPH turns to colorless DPPH-H.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay to determine antioxidant activity

The free radical scavenging activity of methanol extracts of rice was measured by 2,2-dipheyl-1-picryl-hydrazyl (DPPH) using the method described by Choi et al. (2000). The DPPH is stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. The method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical from DPPH-H. Thus antioxidant molecule (AH) can quench DPPH free radical (DPPH$^{\cdot}$) (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them a color less products DPPH-H (2, 2-diphenyl-1-hydrzine, or a substitute analogous hydrazine)

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

Hence, the more rapidly absorbance decreases the more potent antioxidant activity of the extract in term of hydrogen atom donating capacity/ electron transport ability. In this assay, the positive control can be ascorbic acid, gallic acid, quercetin, BHT, rutin or catechin. Molecular mechanisms of DPPH and antioxidant reaction are given in Fig. 2.

Two milliliter of methanol solution of plant extract or standard at different concentration was taken in test tube. 3 ml of methanol solution of DPPH (0.1 mM) was added in to the test tube. The test tube was incubating at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against control. A typical control solution contains all reagents except plant extract or standard solution. The percentage (%) inhibition activity was calculated from the following equation:

$$\% I = \{(A_0-A_1)/A_0\}*100$$

Where,

A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

Then % of inhibition was plotted against blank concentration and from the graph IC_{50} was calculated. (IC_{50} value, the concentration of sample required for 50% scavenging of DPPH free radical are completed)

Results

Phytochemical analysis

Phytochemical screening of various extracts of different rice part revealed the presence of alkaloid, flavonoid, terpenoids, tannins and phytosterols. The results have been summarized in Table 1.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The antioxidant of crud methanol extract (CME) of bran, polishes and unpolished grain of five rice genotypes were evaluated by DPPH radical scavenging assay and shown in Table 2. Lower IC_{50} value indicate highest antioxidant activity where higher are lowest activity. Figure 3 shows the dose response curve of crud methanol extract of different parts of five rice genotype comparing with BHT (standard). Those figures illustrate a significant increase of DPPH radical scavenging ability for increasing concentration for all treatments.

Bran of all genotypes (Kalijira 60.12±0.33, Chinigura 350.1±0.05, Hizoldigha 230.6±0.17, BRRI dhan28 360.4±0.8, BRRI dhan29 340.4±0.75) except Hizoldigha showed greatest antioxidant activity compeering unpolished grain (Kalijira 410.3±0.49, Chinigura 420.3±0.46, Hizoldigha 130.2±0.16, BRRI dhan28 490.8±0.06, BRRI dhan29 460.2±0.74) and polished grain (Kalijira 710.7±0.88, Chinigura 790.5±0.30, Hizoldigha 510.1±0.07, BRRI dhan28 1010.5±1.19, BRRI dhan29 990.0±2.88),where polished grain of all genotypes showed lower performance. Kalijira Bran showed better performance compeering other varieties bran but unpolished and polished grain of Hizoldigha showed better performance to show antioxidant activity.

Discussion

Over the past 20 years a significant amount of research has been directed toward the study of rice because it's several key components exhibit antioxidant properties that could provide a source of natural antioxidant in the prevention of colon cancer, digestive cancers, breast cancer and prostate cancer (Adom and Liu, 2002).

Here the antioxidant activity of bran, polished and unpolished grain of five local and HYV of Bangladesh were examined. These samples showed significant antioxidant activity in a concentration dependent manner through the scavenging of DPPH radical. There are many compounds responsible for antioxidant in rice e.g. Ferulic acid, p-coumaric acid, Vanilic acid, Syringic acid and Gallic acid (Tian *et al.,* 2004, Zhou *et al.,* 2004), Caffeic acid, Sinapic acid, Tricin (flavone) Protocatechuic acid, Esters (6-o-feruloysucrose and 6-o-(e) sinapoylsucrose),

Chlorogenic acid and Hydroxybenjoic acid (Hudson *et al.,* 2000, Tian *et al.,* 2005), Anthocyanins (cyaniding-3-o-β-d-glucoside and peonidin-3-o-β-d-glucoside) (Chen *et al.,* 2006, Zhang *et al.,* 2006, Yawadio *et al.,* 2007), γ-oryzanol and Vitamin E (Tocopherols & Tocotrienols) (Iqbal *et al.,* 2005), Vitamin B, Zinc (USDA National Nutrient Database) etc.

Table 1. Phytochemical analysis of various extracts of different rice parts.

		Solvent	Alkaloids	Saponins	Terpenoids	Glycosides	Flavonoides	Tannins
Kalijira	Bran	Methanol	W>T>D>M>H>C	+	++	-	-	+
		Ethanol	T>W>D>H?M>C	++	++	-	-	+
	Grain	Methanol	W>T>D>M>H>C	++	+	-	+	-
		Ethanol	W>T>D>M>H>C	+	-	-	-	-
Chinigura	Bran	Methanol	T>W>D>H>M>C	-	+	-	-	-
		Ethanol	W>D>T>M>H>C	-	+	-	-	-
	Grain	Methanol	T>W>D>M>H>C	++	+	-	+	-
		Ethanol	T>W>D>M>H>C	+	-	-	-	-
Hizoldigha	Bran	Methanol	T>W>D>H>M>C	-	+	-	+	-
		Ethanol	W>D>T>M>H>C	-	+	-	+	-
	Grain	Methanol	T>W>D>H>M>C	+	+	-	+	-
		Ethanol	W>D>T>M>H>C	+	+	-	+	-
BRRI dhan 28	Bran	Methanol	T>W>D>H>M>C	-	++	-	++	-
		Ethanol	W>D>T>M>H>C	-	++	-	++	-
	Grain	Methanol	T>W>D>M>H>C	++	++	-	+	-
		Ethanol	T>W>D>M>H>C	+	+	-	+	-
BRRI dhan 29	Bran	Methanol	T>W>D>H>M>C	-	++	-	++	-
		Ethanol	W>D>T>M>H>C	-	++	-	++	-
	Grain	Methanol	T>W>D>M>H>C	++	++	-	+	-
		Ethanol	T>W>D>M>H>C	+	+	-	+	-

(+) present, (-) absent, W= Wagner's reagent, T=Tannic acid reagent, D=Dragendroff's reagent, H= Hager's reagent, M= Mayer's reagent, C= Control.

However, throughout history milled rice (white rice or polished rice) has been the major form of consumed rice while the remaining part of the whole rice grain has been discarded or used as animal feed although it has long been considered an excellent source of vitamins and other nutrients. Bidlack (1999) has shown that rice bran may contain over 100 different antioxidants. However result showed that rice bran has high antioxidant activity and comparatively more than grain (both polished and unpolished). Many scientists have reported about antioxidant activity of rice bran. Chotimarkorn et al. (2008) said that the methanol components of the long-grained rice bran extracts might potentially be natural antioxidants. Lloyd *et al.* (2000) also reported that, rice bran contains high amounts of beneficial antioxidants including tocopherols, tocotrienols, and oryzanols. Rao et al. (2010) showed that the crude methanol extract from Njavara rice bran contains significantly high polyphenolic compounds with superior antioxidant activity as evidenced by scavenging of free radicals including DPPH. Chatha *et al.* (2006) demonstrated that rice bran extracts of the Super Kernel variety indigenous to Pakistan are a viable source of natural antioxidants and might be exploited for functional foods and nutraceutical applications.

It is also remarkable that, antioxidants containing level also depend on the type of rice (Gaydou *et al.,* 1980). Although In this experiment five rice genotypes were used but they were different types. Kalijira bran was black in color and its methanol extracts were deep purple while remaining bran was golden or yellowish in color and those methanol extracts were same. Studied results were showed that, antioxidant activity of Kalijira bran was higher than other bran. Pigmented rice bran is a type of rice which may provide additional benefits to human health. Pigmented rice such as black, purple, or red rice is a good source of antioxidants because it contains anthocyanins which are effective free radical scavengers (Romero *et al.,* 2009). Laokuldilok *et al.* (2011) reported that Black rice bran contained gallic, hydroxybenzoic, and protocatechuic acids in higher contents than red rice bran and normal rice bran. Pigmented rice also contains anthocyanins. Anthocyanins are responsible for cyanic color of pigmented rice and are regarded as important nutraceuticals mainly due to their antioxidant effect, which provide a potential to prevent various diseases associated with oxidative stress (Kong *et al.,* 2003). Cyanidin-3-O-b-D-glucopyr-anoside is the most abundant pigment in purple rice (Ryu *et al.,* 1998). It also has been known to have diverse physiological effects protection against cytotoxicity, antioxidative activity (Rossi *et al.,* 2003). Yodmanee *et al.* (2011) and Srisawat *et al.* (2010) also demonstrated that colored rice bran can be promising sources of potential natural antioxidants. Nam *et al.* (2006) said that, with some exceptions, extracts from the pigmented rice seeds had higher antioxidative activity than did the nonpigmented variety.

Table 2. IC_{50} values of DPPH radical scavenging activity of different parts of five rice genotypes.

Treatment	Parts	IC_{50} (µg/ml)
BHT		37.35
Kalijira	Bran	**60.12±0.33** a
	Unpolished grain	410.3±0.49 b
	Polished grain	710.7±0.88 c
Chinigura	Bran	350.1±0.05 i
	Unpolished grain	420.3±0.46 j
	Polished grain	790.5±0.30 k
Hizoldigha	Bran	230.6±0.17 p
	Unpolished grain	**130.2±0.16** q
	Polished grain	510.1±0.07 r
BRRI dhan28	Bran	360.4±0.8 m
	Unpolished grain	490.8±0.06 n
	Polished grain	1010.5±1.19 o
BRRI dhan29	Bran	340.4±0.75 x
	Unpolished grain	460.2±0.74 y
	Polished grain	990.0±2.88 z

(IC_{50} value, the concentration of sample required for 50% scavenging of DPPH free radical are completed)

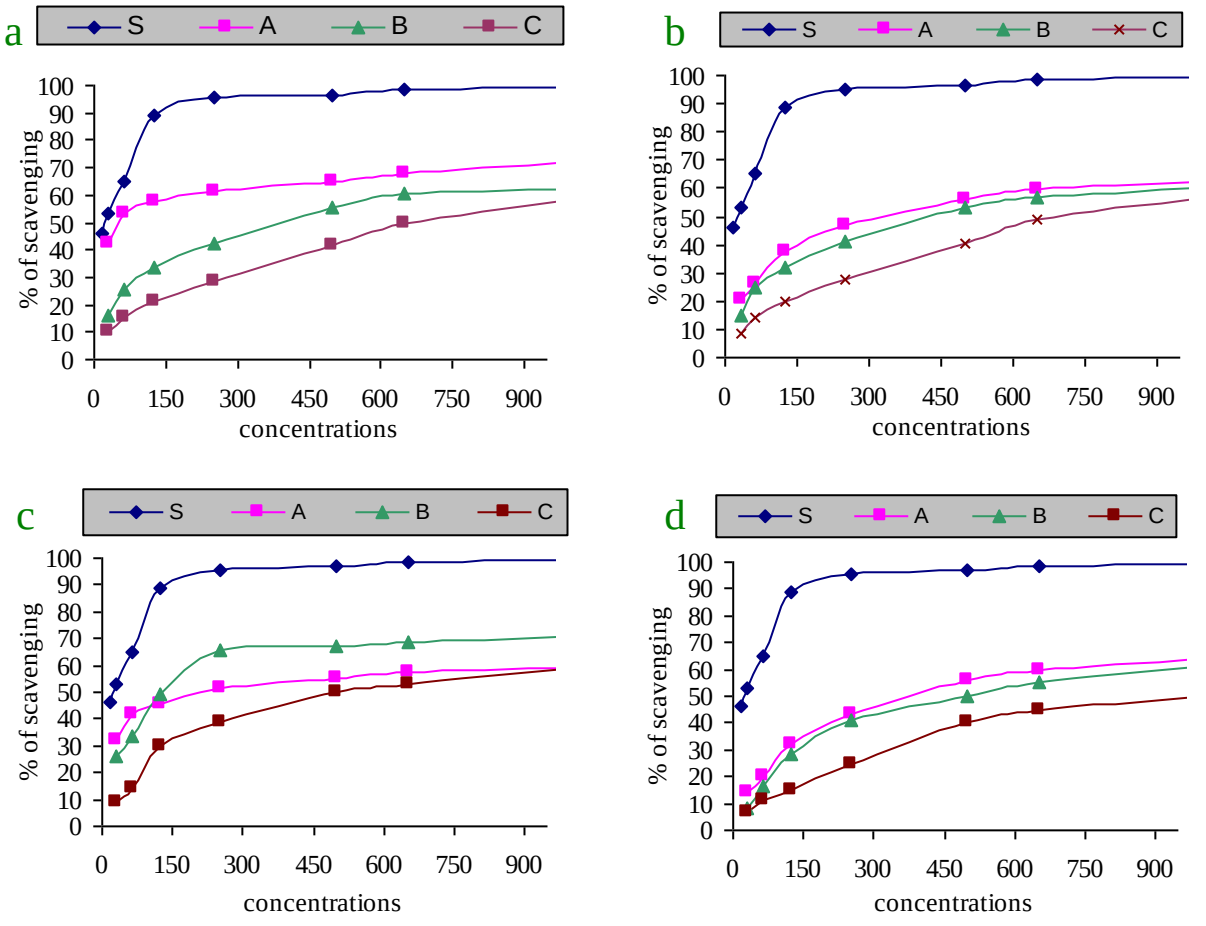
Rice grain is also a rich source of antioxidant compound. Results showed that both unpolished and polished grain contains antioxidant compound while unpolished grain contains significantly higher than polished in all cultivars. Phenolic compounds are one of the more potent antioxidant compounds what are associated with the pericarp of the rice. The concentration of total phenolics in the rice grains has

been positively correlated with the antioxidant activity (Zhang *et al.*, 2006). Walter and Marchesan (2011) reports on different phenolic compounds of rice. Min *et al.* (2012) and Srisawat *et al.* (2010) also reported on antioxidant activity of rice grain. Milling process reduces the concentration of these compounds in grains. The B vitamin and iron are found primarily in the grain and are therefore removed in polishing. It had been estimated that in the 80% to the thiamin is removed (Abbas *et al.*, 2011).

Yafang *et al.* (2011) studied that the size of grain is correlated with phenolic compound. They reports that the smaller grains had higher phenolic content, flavonoid content and antioxidant capacity than the normal and larger grains. Among studied rice cultivars Kalijira and Chinigura is smaller in size and its might be an evidence in favor of containing high level of antioxidants in its. Hizoldigha rice pericarp is red in color what might be the main cause of its high antioxidant activity compeering other.

However, it can be summarized that, all of the studied cultivars are contained antioxidant compounds. Among those Kalijira bran is pigmented (black) and contained higher antioxidant compounds than other bran and Hizoldigha grain covers with red pericarp that possess high antioxidant compound. It is also mentioned that the bran is the more potent part of rice to show antioxidant activity where unpolished grain also more beneficial for containing pericarp and embryo than polished grain.

More research is needed to screen high antioxidant containing rice genotypes and to increase its antioxidant level through genetic manipulations.



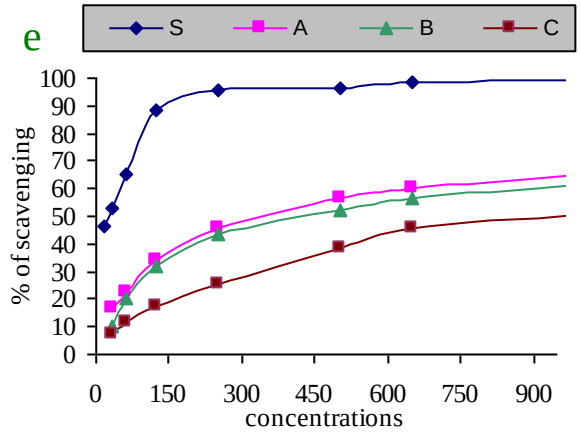


Fig. 3. DPPH radical scavenging activity of a. Kalijira, b. Chinigura, c. Hizoldigha, d. BRRI dhan28 and e. BRRI dhan29.

Where, S= BHT(standard)

A= Bran

B= Unpolished grain

C= Polished grain

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