International Journal of Management, IT & Engineering
Vol. 7 Issue 3, March 2017,
ISSN: 2249-0558 Impact Factor: 7.119
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

ANTIOXIDANT ACTIVITY OF *RHAMNUS PRINOIDES* AND HERKULE HOPS FOR BARLEY MALT BEER PRODUCTION IN ETHIOPIA

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ABSTRACT

The effects of antioxidant activity of the Gesho (Rhamnus prinoides) extract, its residual and Herkule were evaluated by using 2, 2-diphenyl-2- picryl-hydrazine (DPPH) assay. Extraction of well vented and dried samples of R. prinoides was performed by socking in 97% ethanol as an extracting agent. Free radical scavenging activities of R. prinoides extract and Herkule was read spectrophotometrically and inhibition of free radical DPPH in percent (I %) was calculated and compared against the standard ascorbic acid. From the study Herkule, R. prinoides and ascorbic acid showed a maximum radical scavenging as 80.87, 81.148 and 93.77% at concentrations of 0.24mg/ml, 0.24mg/ml and 0.160mg/ml, respectively. Inhibitory concentration (IC_{50}) was calculated for herkule, R. prinoides extract and ascorbic acid to be 5.2, 5.2 and 0.2 mg/ml, respectively. Moreover, the effect of different concentration of R. prinoides extract with DPPH free radical on absorbance at 517 nm is similar to that of standard ascorbic acid with varying degree of inhibition and comparable to that of the Herkule activity. Thus, this study suggests that the ethanolic extract of R. prinoides has a good antioxidant activity like Herkule as a potential source of natural antioxidant. This study indicated that R. prinoide plant is a promising source of antioxidants for utilization in beer brewery fields as bittering agent.

Keywords: Antioxidant activity; DPPH assay; Herkules; Rhamnus prinoides; Scavenging activity

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1. INTRODUCTION

Over the last several decades, scientists have discovered that the body's formation of unstable oxygen molecules called free radicals is unavoidable and almost every cell produces tens of thousands of them each day. We are also exposed to free radicals in the environment. Thus, free radicals have endogenous and exogenous sources of origin. Our antioxidant systems are not perfect so as we age, cell constituents are constantly damaged by oxidation which may lead to diseases and therefore should be mopped up by antioxidants. It is established that body's own antioxidants are insufficient and should be restored by external sources. Food and beverages are major sources of antioxidants containing phenolic or polyphenolic nucleus with widely demonstrable activity in vitro (Halliwell and Gutteridge, 1999). Times immemorial, beer has been known for its prophylactic, therapeutic and palliative properties (Rice Evans, 2000; Andersen et al., 2000).

Beer is essentially prepared from malted Barley and Hops which contribute several constituents in it. Barley mainly contributes the sugars, amino acids, proteins, Polyphenols. Its malted form is the chief source of carbohydrates fermented by *Saccharomyces cerivisiae* converting it into ethanol and CO_2. While Hops added during the later stage of brewing is the chief source of antioxidants activity (AO). The AO activity of the beer is mainly due to the presence of Polyphenols, chalcones, flavonoids, catechins, proanthocyanidins and bitter compounds e.g., humulones, lupulones, etc. (Wolfhart,1993; Stevens et al., 1998). Besides AO, hops in beer also possess sedative, chemo preventive, drug modulating and phytoestrogenic properties (Hopfenzapfen, 2000; Gerhauser et al., 2002).

Food and beverages including beer, since natural sources of AO are far more superior for better utilization than isolated preparations (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Beer is an important and popular beverage liked by the majority and needs to be elucidated for its AO property and bioavailability. The brewing industry is becoming more competitive and constantly looking for ways to improve beer quality and reduce manufacturing costs in the worldwide. In tropical countries, Hops has been imported from other temperate countries and this involves the expenditure of scare foreign exchange. In the world there are five top hop producing countries. These are German, USA, Ethiopia, China and Czech Republic

(FAOSTAT data, 2010). Ethiopia is one of top five hop growing countries in the world, but nowadays, Beer in Ethiopia is produced with imported hop as bittering agent. Since it is imported it creates a pressure on its foreign currency. The total beer consumption in Ethiopia is around 12 million hectoliter of beer per year to produce this amount overall cost for hops is about 479.27 million birr per year, from which overall hop costs about 127 million birr per year is due to transport costs per year(MOIN., 2016).

The present finding was conducted to evaluate the effects of antioxidant activity of the Gesho (Rhamnus prinoides) ethanolic extract, its residual and imported hops (Herkule) for effective utilization in beer brewery fields as bittering agent.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Rhamnus prinoides L .Herit, the fresh plant leaves and a stem of Gesho (*Rhamnus prinoides*) was collected from Debr Berhan Region of Ethiopia, and the extraction would be carried out at Wood Technology Research Center of Ethiopia Environmental and Forest Research Institute (EEFRI). The moisture of freshly harvested sample of Gesho leaves have moisture content of 75 -80% are shown in Figure 1.

Figure 1. Leaves of Gesho (*R. prinoides).*

Drying was performed in an open air protected from direct exposure to sunlight for five days to moisture content of 10.4%. The dried materials were powdered using Wiley mill as shown in Figure 2.

Figure 2. Mill (Retsch SR200).

Cold extraction of dried and milled sample of Gesho was performed with 97% of ethanol by socking for 72hr. The experiments done for production of barley malt beer using *R. Priniodes* and imported hops based on European brewing technology and the comparative analysis of R. prinoides with hops on the beer production was done.

2.2. Extraction process of *Rhamnus prinoides*

Extraction of well vented and dried samples of Gesho was performed by socking in 97% ethanol as an extracting agent. A weight of 750g of the dried and powdered *R. prinoides* leaves with stem put in 2000 ml Erlenmeyer flask was soaked in 1500ml of 97% ethanol for a period of about 72 hours with by shaking on a shaker Hy-5A Manoeuvre (Figure 3). Fresh solvent was used in every 12 hr socking extract was filtered through a medium density filter paper fitted in Buchner funnel. And the extract of *R. prinoides* i.e. ethanol extracted sample was concentrated using rotary evaporator.

Figure 3. Hy-5A mayoeuvre style vibrator (shaker)

2.3. Antioxidant activity determination of *Rhamnus prinoides* and hops (Herkule)

The antioxidant activity of the *R. prinoides* ethanolic extract, residual and Herkule using ethanol as a solvent was estimated using the method of Kirby and Schmidt (2004) with slight

modification. The method of Kirby and Schmidt uses DPPH (2,2-diphenyl-1-picrylhydrazine) as a free radical. For measuring free radical scavenging ability of a sample using this method, the methods are grouped in to two according to the chemical reactions involved: hydrogen atom transfer reaction-based methods and single electron transfer reaction-based methods. This radical shows a strong absorption maximum at 517 nm (purple), in the presence of antioxidants, the color turns yellow as shown in Figure 4.

Accordingly an amount of 0.004% DPPH and 0.3 mg/ml of standard ascorbic acid was prepared in a 98% methanol solvent and 50 mg/ml of *R. prinoides* extract, *R. prinoides* residue and Herkule extract were also prepared using ethanol (97%) as a solvent. Then 20μl, 40μl, 60μl, 80μl, 100μl, 120μl, 140μl, 160μl, 180μl , 200μl and 240 μl sample was taken from 50 mg/ml of *R. prinoides, R. prinoides* residual and Herkule stock solution , 98μl, 96μl, 94μl, 92μl, 90μl, 88μl, 86μl, 84μl, 82μl , 80μl and 76 μl of the methanol and 4ml of 0.004% of DPPH free radical solution were added to each sample in the different test tubes and mixed using a vortex mixer for 20s. All test tubes were then incubated for 30 minutes in the dark cabinet at room temperature. This makes the reaction to happen and prevents the absorption of light by DPPH that may interfere with the actual absorbance of the solution. The same procedure was repeated for standard by replacing the *R. prinoides* extracted, residual and Herkule sample with equal amount of 0.3 mg/ml for ascorbic acid. Control sample (without extracted, residual, Herkule sample and ascorbic acid) that is blank was run in parallel.

Finally, free radical scavenging activities was read spectrophotometrically by monitoring the decrease in absorbance at 517nm using high performance UV-Vis spectrophotometer and Cuvette(1-cm,quartz) inhibition of free radical DPPH in percent (I %) was calculated as follows.

$$I\% = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) X100$$

Where:

- $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound)
- A_{sample} is the absorbance of the test compound.

Figure 4. In the presence of antioxidant colour turns to yellow.

3. RESULTS AND DISCUSSIONS

3.1. Antioxidant activity of *R. prinoides* extracted, residual and Herkule

Antioxidant activity of *R. prinoides* extracted was evaluated by its free radical scavenging activity on stable DPPH radical. Ascorbic acid was used as standard and a control sample was run in parallel.

Table 1. Inhibition effect of ascorbic acid as standard on DPPH assay.

Ascorbic acid conc. (mg/ml)	Absorbance at 517 nm	Inhibition effect (%)
0.000	0.722±0.001	0.00±0.00
0.020	0.546±0.012	24.38±1.66
0.040	0.411±0.044	43.07±0.01
0.060	0.32o±0.052	55.68±0.06
0.080	0.143±0.002	80.19±0.02
0.100	0.048±0.003	93.35±0.004
0.120	0.051±0.008	92.94±0.10
0.140	0.047±0.002	93.49±0.04
0.160	0.045±0.001	93.77±0.08
0.180	0.045±0.000	93.77±0.00
0.200	0.045±0.000	93.77±0.00
0.240	0.050±0.000	93.07±0.00

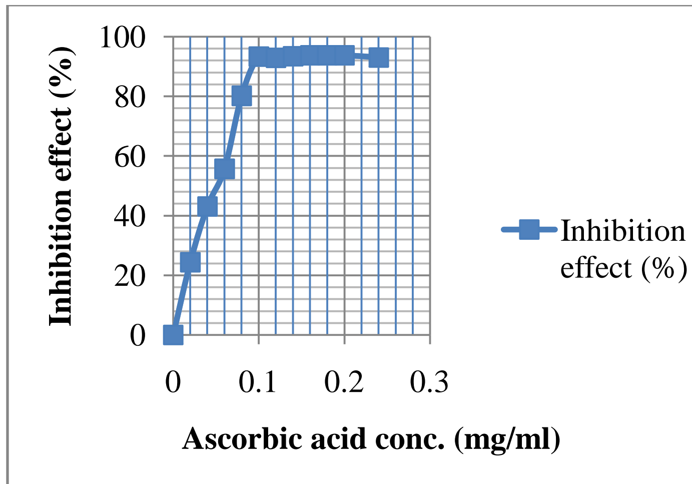

Figure 5. Inhibition effect of different concentration of ascorbic acid on DPPH free radical at 517 nm

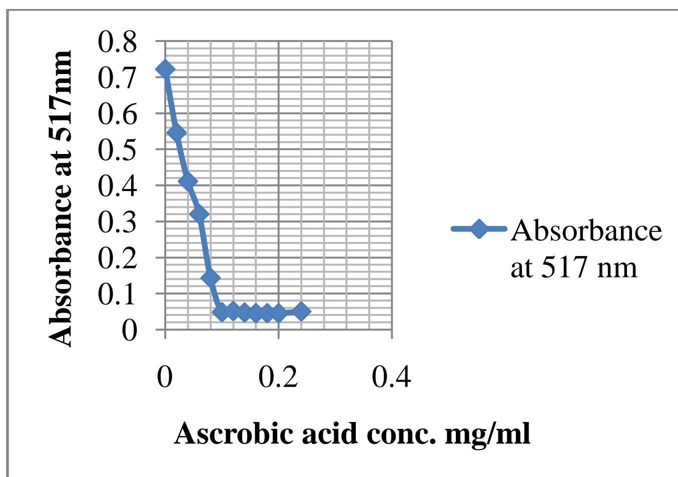

Figure 6. Effect of different concentration of ascorbic acid with DPPH free radical on absorbance at 517 nm.

Table 1 presented that ascorbic acid is the strong antioxidant having maximum inhibition effect of 93.77% and the inhibition effect increase in the first phase until the maximum value of inhibition effect is achieved and constant with further addition of ascorbic acid (Fig. 5).

Table 1. Inhibition effect of hops (Herkule) on DPPH assay.

Herkule conc. (mg/ml)	Absorbance at 517nm	Inhibition effect (%)
0.000	0.915±0.013	0.00±0.00
0.020	0.798±0.004	11.68±0.437
0.040	0.664±0.002	27.43±0.219
0.060	0.54±0.028	40.98±3.06
0.080	0.448±0.007	51.04±3.716
0.100	0.364±0.004	60.22±0.437
0.120	0.308±0.000	66.34±0.000
0.140	0.267±0.012	70.82±1.311
0.160	0.230±0.01	74.86±1.093
0.180	0.207±0.001	77.38±0.109
0.200	0.190±0.001	79.23±0.109
0.24	0.175±0.001	80.87±0.109

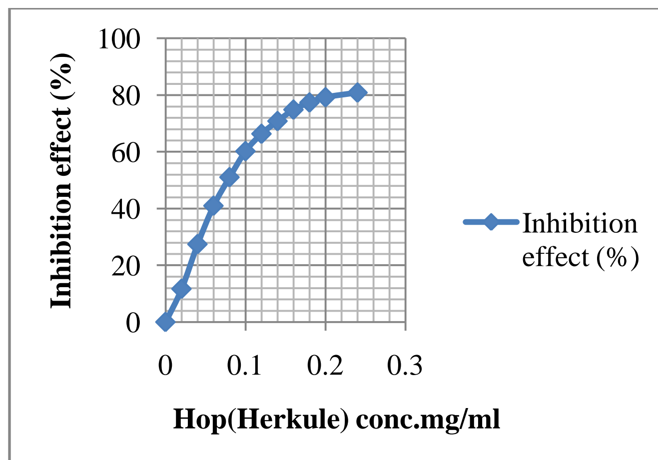

Figure 7. Scavenging effect of different concentration of hop (herkule) on DPPH free radical at 517 nm.

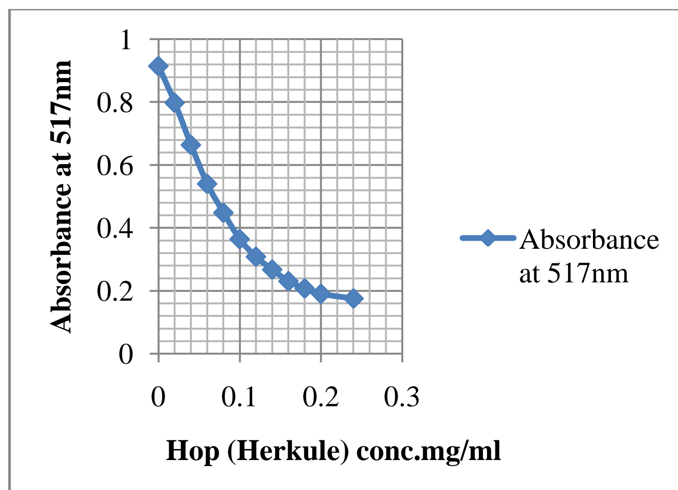

Figure 8. Effect of different concentration of hop (herkule) with DPPH free radical on absorbance at 517 nm

Table 2 was presented hop (herkule) is strong antioxidant having maximum inhibition effect of 80.87% at concentration of 0.24 mg/ml and the inhibition effect increase rapidly in the first phase and nearly constant with further increase in concentration of herkule (Fig. 7).

The nature of the graph as shown in Fig. 7, the inhibition effect versus the concentration of herkule increases in the first phase until it reaches the maximum, and then there is no change in effect with an increase in concentration of the herkule. In Figure 8, the AO of the hop graph trend is similar to that of the standard sample of ascorbic acid with varying inhibition degree (Fig. 6). The effect of antioxidant on DPPH radical scavenging is due to their hydrogen donating ability. When a solution of DPPH is mixed with that of a substance, it can generate a hydrogen atom. This results in the reduced form of DPPH- H (non-radical) with change of the violet color to yellow when it reaches its maximum inhibition effect. DPPH scavenging activity is usually presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Therefore, extract concentrations providing 50% inhibition (IC_{50}) were calculated using the data plotted in Fig. 5 and 7 Lower IC_{50} value reflects better DPPH radical-scavenging activity and has strong antioxidant potential.

This study compared the free radical scavenging activity of imported hops (Herkule) against the standard ascorbic acid. The scavenging of DPPH radical was determined by level of reduction in

absorbance at 517 nm, which is dependent to concentrations (Fig. 6 and 8). Herkule and ascorbic acid showed a maximum radical scavenging as 80.87% and 93.77% at concentrations of 0.24mg/ml and 0.160mg/ml, respectively. Inhibitory concentration (IC_{50}) was calculated for herkule and ascorbic acid to be 5.2 and 0.2 mg/ml, respectively. This was due to the fact that Hops added during the later stage of brewing are the chief source of AO compounds such as Polyphenols, chalcones, flavonoids, humulones, and lupulones. Different reports indicate that the activity of the beer is mainly due to the presence of Polyphenols, chalcones, flavonoids, catechins, proanthocyanidins and bitter compounds e.g., humulones, lupulones, etc. (Wolfhart,1993; Stevens et al., 1998).

Besides AO, hops in beer also possess sedative, chemo preventive, drug modulating and phytoestrogenic properties (Hopfenzapfen, 2000; Henderson et al., 2000; Miranda et al., 2000b).

Table 2. Inhibition effect of Rhamnus prinoides extracted on DPPH assay.

RPE conc. (mg/ml)	**Absorbance at 517nm**	**Inhibition effect (%)**
0.00	0.891±0.011	000±0.00
0.02	0.792±0.006	11.110±0.673
0.04	0.697±0.004	21.770±0.449
0.06	0.631±0.005	29.181±0.561
0.08	0.558±0.003	37.374±0.337
0.10	0.494±0.004	44.557±0.449
0.12	0.444±0.005	50.168±0.561
0.14	0.373±0.000	58.137±0.000
0.16	0.320±0.002	64.085±0.224
0.18	0.270±0.006	69.697±0.673
0.20	0.243±0.004	72.727±0.449
0.24	0.165±0.005	81.148±0.561

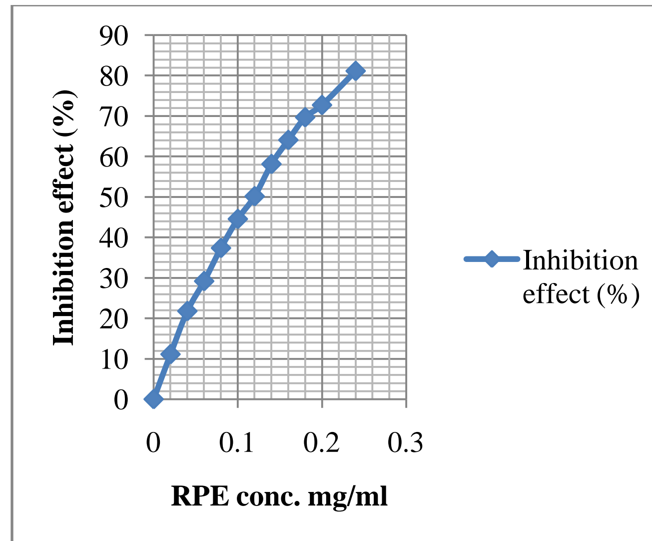

Figure 9. Scavenging effect of different concentration of hop (herkule) on DPPH free radical at 517 nm.

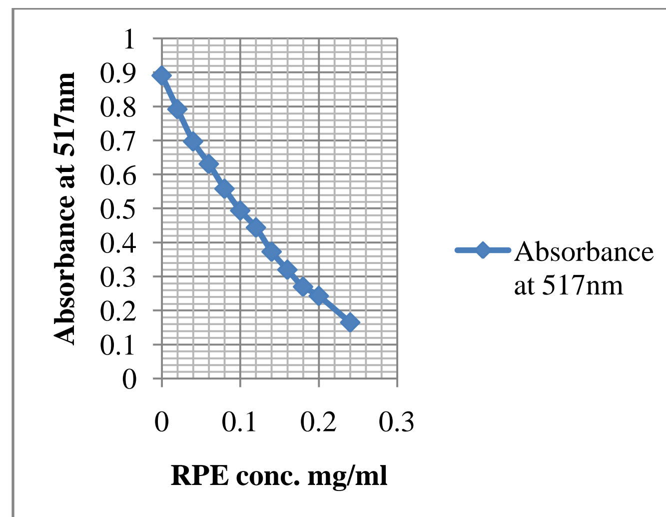

Figure 10. Effect of different concentration of *R. prinoides* extracted with DPPH free radical on absorbance at 517 nm

From above Table 3 shows *R. prinoides* extract is strong antioxidant having maximum inhibition effect of 81.148% at concentration of 0.24 mg/ml and the inhibition effect increase nearly linear with further increase in concentration of *R. prinoides* extracted as shown in Fig. 9. The trend of the graph in Fig. 10 is similar to that of the standard ascorbic acid (Fig. 6) with varying degree of inhibition and comparable to that of the imported hop activity.

This study evaluated the free radical scavenging activity of *R. prinoides* extracted and ascorbic acid. The scavenging activity of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Fig. 8 and10). *Rhamnus prinoides* extracted and ascorbic acid showed a maximum radical scavenging as 81.148 and 93.77 % at concentrations of 0.24mg/ml and 0.160 mg/ml, respectively. Inhibitory concentration (IC_{50}) was calculated for *R. prinoides* extract and ascorbic acid to be 5.2 and 0.24 mg/ml, respectively. This was due to the fact that *R. prinoides* extract contains antioxidant compound such as polyphenol, Geshodin, etc (Abegaz and Kebede 1995).

Ascorbic acid, Herkule and Gesho are considered to be three of the most oxdatively stable AO in beer. Considering that the antioxidant activity of the *R. prinoides* ethanolic extract is mainly due to its polyphenol, humulone, cohumulone, etc.

Table 3. Inhibition effect of *R. prinoides* residual on DPPH assay.

RPR conc. (mg/ml)	Absorbance at 517nm	Inhibition effect (%)
0.00	0.936±0.000	0.000±0.00
0.02	0.834±0.002	10.897±0.214
0.04	0.722±0.008	22.86±0.855
0.06	0.638±0.000	31.838±0.000
0.08	0.573±0.003	38.782±0.321
0.10	0.498±0.017	46.795±1.816
0.12	0.423±0.012	54.808±1.282
0.14	0.356±0.015	61.966±3.788
0.16	0.300±0.004	67.949±0.427
0.18	0.245±0.001	73.825±0.106
0.20	0.199±0.003	78.739±0.321
0.24	0.136±0.006	85.470±0.641

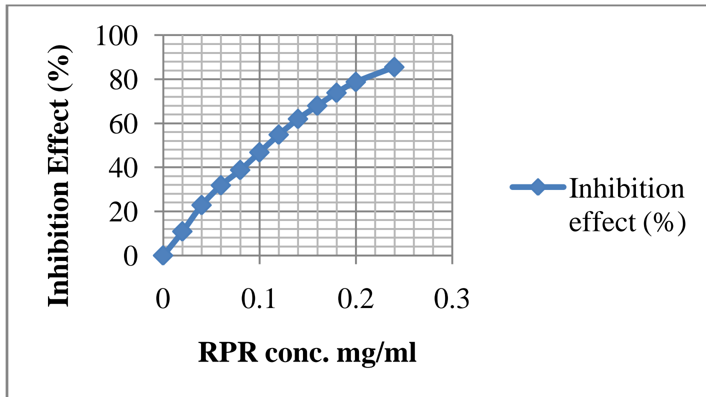

Figure 1. Scavenging effect of different concentration of RPR on DPPH free radical at 517 nm

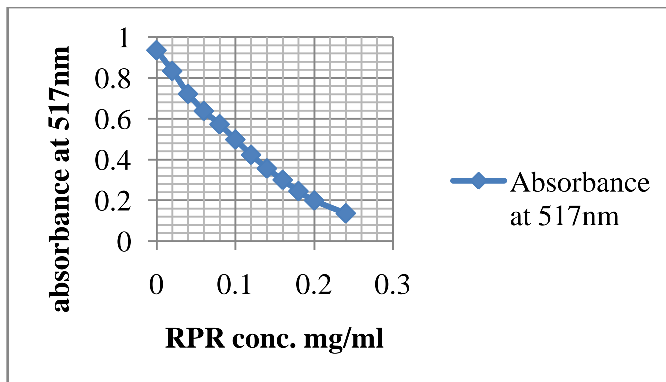

Figure 12. Effect of different concentration of RPR with DPPH free radical on absorbance at 517 nm.

Table 4 presented that *R. prinoides* residual has a strong antioxidant activity having maximum inhibition effect of 85.47 at concentration of 0.24 mg/ml and the inhibition effect increase with further increase in concentration of *R. prinoides* residual similar to *R. prinoides* extracted.

The nature of the graph as shown in Fig. 11, the inhibition effect versus the concentration of *R. prinoides* residual (RPR) increases with increase in concentration of the RPR having a linear functionality. The graph trend in Fig. 11 is unlike that of the standard ascorbic acid graph trend in fig. 5 where the ascorbic acid has two regions: an increasing in inhibition till it reaches 93.77% and an asymptotic region to the maximum inhibition with further increase in concentration. .

This study evaluated the free radical scavenging activity of *R. prinoides* residual and ascorbic acid. The scavenging of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Fig. 10 and 12). *Rhamnus prinoides* residual and ascorbic acid showed a maximum radical scavenging as 85.47 and 93.77 % at concentrations of 0.24 and 0.16 mg/ml, respectively. Inhibitory concentration (IC_{50}) was calculated for *R. prinoides* residual and ascorbic acid to be 5.2 and 0.24 mg/ml, respectively.

4. CONCLUSIONS

This paper presents antioxidant activities of bitter effects (extracts) derived from *R. prinoides* (local Gesho) and Herkule (imported) were evaluated by its free radical scavenging activity on stable DPPH assay since bitter taste and antioxidant activities are suitable for application in brewing. The results of the current study showed that all the extract of *R. prinoides* and Herkule exhibited different extent of antioxidant activity. Thus, this study suggests that the ethanolic extract of Gesho has a good antioxidant activity like Herkule as a potential source of natural antioxidant. The finding of this study supports that *R. prinoides* plant is a promising source of antioxidants for utilization in beer brewery fields as bittering agent.

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