



## Standardization of Protocols for Extraction of *Aloe vera* and Cinnamon Bark Extracts

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### ABSTRACT

The present study was envisaged to optimize the extraction protocols viz. time, temperature and solvent concentration for extraction of phyto-extracts from *aloe vera* and cinnamon bark based on antioxidant efficacy *in-vitro*. These were optimized using Box-Behnken Design (BBD) and completely randomized design (CRD) of Response Surface Methodology (RSM). A total number of 17 different combinations of time, temperature and ethanol concentration in BBD and 13 different combinations of time, temperature in CRD were considered for optimizing extraction protocols for *aloe vera* and cinnamon respectively. The *in-vitro* 1, 1 diphenyl-2picrylhydrazyl (DPPH % inhibition) and 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ABTS<sup>+</sup> radical scavenging activity of aqueous extract of cinnamon bark was recorded highest for the combination of 99% concentration at 80°C temperature for 3h amongst all 13 runs. The 90% ethanolic extract of *aloe vera* and cinnamon bark exhibited higher *in-vitro* antioxidant ability than aqueous extract at time, temperature combination of 15 min for 65°C and 9h for 60°C respectively.

**Keywords:** *Aloe-vera*, cinnamon bark, extraction, free radical scavenging ability

Lipid oxidation and oxidative rancidity still remains a major concern in meat industry as meat is inherently low in antioxidants. Thus meat and meat products are more prone for spoilage and quality deterioration due to lipid peroxidation and oxidative rancidity. These changes have adverse effect on sensory (mostly colour and flavour) and nutritional properties of meat and meat products. Lipid oxidation leads to lower cooking yield, discolouration, production of compounds emanating off odour as well as exerting ill effect on consumer's health. To overcome these problems, meat industry has been using mostly synthetic antioxidants during processing. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and Tert-butyl hydroxyl quinone (TBHQ) are the most common synthetic antioxidant used in food industry. The regular consumption of

these synthetic antioxidants in large amounts has been associated with carcinogenicity and other toxic effects. This resulted in the demand for natural antioxidants to be used by meat industry by replacing synthetic antioxidants. These natural antioxidants are safe, cheaper and present in large quantities in various plants in the form of various bioactive compounds as alkaloids, flavonoids etc. These bioactive compounds can be extracted and used in meat industry. Various phyto-extracts have been prepared using different solvents and extraction methods. *Aloe vera* and cinnamon extracts have been reported to exhibit similar or better antioxidant properties compared to some synthetic antioxidants such as TBHQ.

*Aloe vera* (*Aloe barbadensis* Miller) is a perennial succulent xerophyte, which develops water storage tissue

in the leaves to survive in dry areas of low or erratic rainfall. *Aloe vera* is the most commercialized *aloe* species, and processing of the leaf pulp has become a worldwide industry. In the food industry, it has been used as a source of functional foods and as an ingredient in other food products, for the production of gel-containing health drinks and beverages (Hamman, 2008). Anthraquinones isolated from the exudates of *aloe vera* have shown wide antimicrobial activity, antiviral and virucidal effects (Alves *et al.*, 2004). The activity of *aloe vera* gel against both Gram-positive and Gram-negative bacteria had also been documented (Habeeb *et al.*, 2007).

Cinnamon (*Cinnamomum zeylanicum* Blume), widely used spice, have several applications in perfumery, flavouring and pharmaceutical industries. Although, chemical constituents of leaf and bark essential oils of cinnamon have been studied (Raina *et al.*, 2001; Simic *et al.*, 2004; Jayaprakash *et al.*, 1997). Cinnamon contains 0.5 to 1.0% volatile oil composed mainly of cinnamyldehyde (50.5%), eugenol (4.7%), cinnamic acid, methoxycinnamaldehyde (MOCA) and cinnamyl acetate (8.7%) (Raina *et al.*, 2001). Eugenol is well known for its analgesic, local anaesthetic, anti-inflammatory and anti-bacterial effects. It belongs to the class of essential oils that is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), United State of America (USA). Phenolic antioxidants are soluble in lipids by virtue of the hydroxyl group present in chemical structure, react with cellular membrane of micro-organism impairing its integrity and functionality (Raccach, 1984). Cinnamon have a wide spectrum of actions ranging from antibacterial, anti-viral, anti-fungal and anti-protozoal to beneficial effects on cardiovascular and immune system (Harris *et al.*, 2001).

Due to variations of various natural sources of phenolic compounds in their compositions, structure and physico-chemical properties, designing a universal extraction protocols for various phyto-extracts proves a cumbersome process. Thus a specific extraction procedure should be designed and optimized for each phyto-extracts (Contini *et al.*, 2008; Thoo *et al.*, 2010). The most commonly used extraction media for extracting phyto-extracts for food including meat are ethanol and water because of environment friendly nature and non-toxic effect on human health.

Thus present study was conducted to optimize the extraction protocols of *aloe vera* and cinnamon bark based on their antioxidant efficacy *in-vitro*.

## MATERIALS AND METHODS

### Source of phyto-extracts

*Aloe vera* powder and cinnamon was purchased from local market of Ludhiana, Punjab India. These were carefully cleaned and dried in hot air oven at  $45\pm2^{\circ}\text{C}$  for 2 h. The cinnamon barks were ground mechanically in a domestic grinder (Inalsa, Taureg Marketing Private Limited, Mumbai, India) and sieved into mean particle size from 0.25 to 1.00 mm using ASTM standard sieves for 10 to 15 min. The samples were stored in moisture proof PET (polyethylene terephthalate) at  $-70^{\circ}\text{C}$  till used. The samples were ground within 3 min. using a laboratory ground mill immediately prior to extraction to minimize loss of volatiles compounds.

### Preparation of phyto-extracts

In the present study, ethanol and water were used as the extraction media because of their environmentally friendly effects and non-toxicity for human health. *Aloe vera* powder and cinnamon powder (50 g) was added to a glass thimble, which was placed inside a glass soxhlet apparatus. This was attached to a condenser and a pre-weighed 500 ml round-bottomed flask (quick-fit, UK) containing 200 ml solvent (distilled water or ethanol) and a few anti-bumping granules. The apparatus was placed on an electric mantle. According to response surface methodology (RSM), three variants viz. time, temperature and solvent concentration were used (Table 1, 2, 3 and 4). The solvents were distilled off in a rotary vacuum evaporator (RE-300 Yamato rotary evaporator, Japan) at different time-temperature combination on 210 rpm, leaving the extract in the flask. This process was carried out using aqueous and ethanol solvents for *aloe vera* and cinnamon bark, respectively. Extracts were filtered through Whatman's filter paper 1 and liquid extract was poured in glass petri-plates. These petri-plates were put into incubator for 36-48 h for drying. Dry extract from glass petri plates was collected and stored in amber coloured bottles at  $4\pm1^{\circ}\text{C}$  till further use.

**Table 1:** Various combination of time, temperature and aqueous concentration for the preparation of extract from *aloe vera* powder using Response Surface Methodology (RSM)

Run	Factor 1 (Time-Min)	Factor 2 (Temp-°C)	Factor 3 (Conc %)
1	25	65	99
2	25	65	99
3	25	43.78	99
4	25	65	99
5	39.14	65	99
6	25	65	99
7	35	80	99
8	35	80	99
9	15	80	99
10	10.85	65	99
11	25	65	99
12	15	50	99
13	25	86.21	99

**Table 3:** Various combination of time, temperature and aqueous concentration for the preparation of extract from cinnamon powder using Response Surface Methodology (RSM)

Run	Factor 1 (Time-h)	Factor 2 (Temp-°C)	Factor 3 (Conc%)
1	9	40	99
2	10.24	60	99
3	6	60	99
4	1.75	60	99
5	6	31.71	99
6	3	40	99
7	6	60	99
8	9	80	99
9	6	88.82	99
10	6	60	99
11	6	60	99
12	3	80	99
13	6	60	99

**Table 2:** Various combination of time, temperature and ethanol concentration for the preparation of extract from *aloe vera* powder using response surface methodology (RSM)

Run	Factor 1 (Time-Min)	Factor 2 (Temp-°C)	Factor 3 (Ethanol %)
1	25	65°C	60%
2	25	50°C	90%
3	35	80°C	60%
4	25	65°C	60%
5	25	50°C	30%
6	15	50°C	60%
7	35	50°C	60%
8	15	65°C	30%
9	25	65°C	60%
10	35	65°C	30%
11	25	65°C	60%
12	15	65°C	90%
13	35	65°C	90%
14	25	65°C	60%
15	25	80°C	30%
16	15	80°C	60%
17	25	80°C	90%

**Table 4:** Various combination of time, temperature and ethanol concentration for the preparation of extract from cinnamon bark using response surface methodology (RSM)

Run	Factor 1 (Time-h)	Factor 2 (Temp-°C)	Factor 3 (Ethanol %)
1	3	60 °C	70%
2	6	80 °C	70%
3	3	40 °C	80%
4	6	60 °C	80%
5	9	80 °C	80%
6	6	60 °C	80%
7	6	80 °C	90%
8	6	40 °C	90%
9	6	60 °C	80%
10	3	60 °C	90%
11	6	40 °C	70%
12	3	80 °C	80%
13	6	60 °C	80%
14	6	60 °C	80%
15	9	60 °C	70%
16	9	60 °C	90%
17	9	40 °C	80%

## Experimental design

Box-Behnken Design (BBD) and completely randomized design (CRD) of Response Surface Methodology (RSM) was employed to identify optimum levels of three variables viz. solvent concentration (%), extraction temperature (°C) and extraction time. Various combination of time, temperature and solvent (ethanol) concentration for the preparation of extract from *aloe vera* powder and cinnamon bark are presented in Table 1-4.

## Antioxidant efficacy

Antioxidant efficacy of various phyto-extracts were assessed by ability to scavenge 1, 1 diphenyl-2picrylhydrazyl (DPPH) radical, ABTS<sup>+</sup> radical scavenging activity and total phenolics. The ability to scavenge 1, 1 diphenyl-2picrylhydrazyl (DPPH) radical was estimated following the method of kato *et al.* (1988) with slight modifications. The absorbency in time t=0 min ( $t_0$ ) was measured at 517 nm using a UV-VIS spectrophotometer (Elico India Limited, Mumbai). The sample tubes were also incubated at room temperature under dark for measurement of absorbency in time t=20 min. ( $t_{20}$ ). Ethanol was used as blank sample. The free radical scavenging activity was calculated as a decreased of absorbency from the equation:

$$\text{Scavenging activity (\% inhibition)} = 100 - (at_{20}/at_0) \times 100$$

The spectrophotometric analysis of ABTS<sup>+</sup> radical scavenging activity was determined according to method of ABTS also a relatively stable free radical (Shirwaikar *et al.*, 2006). This method is based on the ability of antioxidants to quench the long-lived ABTS<sup>+</sup> radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of standard antioxidants. The stock solution was diluted with ethanol to an absorbance of 0.70 at  $t_0$  (t=0 min) and equilibrated at 30°C exactly 6 min after initial mixing. About 2 ml of ABTS<sup>+</sup> working standard solution was mixed with 1ml of extract and absorbency was measured after 20 min ( $t_{20}$ ) at 734 nm. The ABTS<sup>+</sup> activity was calculated by using formula:

$$\text{ABTS}^+ \text{ activity (\% inhibition)} = [(0.7 - at_{20})/0.7] \times 100.$$

The total phenolic content was quantified by Folin-Ciocalteau's reagent and was expressed as gallic acid equivalents (Yuan *et al.*, 2005). For preparation of sample

extract same procedure as mentioned for DPPH was followed. A 100 µl of extract (250 ppm concentration) was mixed with 2 ml of 2.0% sodium carbonate buffer and incubated at room temperature for two min. The amount of total phenolics was determined by external standard method substituting respective absorbance in the linear regression formula [ $y = 9207x + 241.55$  and  $r^2 = 0.9949$ ; where,  $y$ = gallic acid concentration,  $x$ = absorbance or (sample reading- blank reading) and  $r^2$ =correlation coefficient] after calibration of standard curve of pure gallic acid.

Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, IL USA) software package as per standard methods (Snedecor and Cochran 1994). The data were subjected to statistical analysis by analysis of variance (ANOVA) and Duncan's multiple range test.

## RESULTS AND DISCUSSION

The perusal of results showed a dose dependent antioxidant activity of phyto-extracts. Among various factors contributing to the efficiency of the solvent extraction process and the recovery of antioxidant compounds from natural materials, solvent concentration, extraction time, extraction temperature considered the main factors influencing antioxidant efficiency of extract.

### Antioxidant efficacy of ethanolic and aqueous extracts of *aloe vera*

The results for antioxidant activity of aqueous and ethanolic extract of *aloe vera* obtained by various combinations of extraction time (15, 25 and 35 min.), extraction temperature (50°C, 65°C and 80°C) in ethanol concentration (30%, 60% and 90%) and aqueous concentration (99%) are presented in Table 5-6 respectively.

DPPH free radical scavenging activity is due to hydrogen donating ability; the more the number of hydroxyl groups, the higher the possibility of free radical scavenging ability (Chen and Ho, 1995). ABTS decolorization assay is an excellent tool for determining antioxidant activity of hydrogen denoting antioxidants. The *in-vitro* antioxidant activity of ethanolic extract of *aloe vera* as observed by radical scavenging activity (DPPH and ABTS) and total phenolics, was recorded highest ( $p < 0.05$ ) for a combination of 90% ethanol solvent at 65°C temperature

**Table 5: Antioxidant activities of ethanolic aloe-vera extract (Mean±SE)\***

Sl. No.	Time (Min)	Temp (°C)	Conc (%)	DPPH (% Inhibition)	ABTS (% Inhibition)	Total Phenolics (GA Equivalent mg/g)
1	25	65	60	43.6±2.63 <sup>d</sup>	62.35±1.43 <sup>ab</sup>	2.62±0.08 <sup>ab</sup>
2	25	50	90	45.38±2.39 <sup>d</sup>	69.21±2.28 <sup>b</sup>	2.68±0.01 <sup>abcd</sup>
3	35	80	60	41.15±1.97 <sup>cd</sup>	66.57±1.23 <sup>b</sup>	3.10±0.07 <sup>bcdx</sup>
4	25	65	60	43.70±1.52 <sup>d</sup>	62.37±1.64 <sup>ab</sup>	2.64±0.09 <sup>abc</sup>
5	25	50	30	33.77±1.41 <sup>ab</sup>	68.48±1.66 <sup>b</sup>	2.69±0.07 <sup>abcd</sup>
6	15	50	60	32.35±1.41 <sup>ab</sup>	66.45±1.90 <sup>b</sup>	3.17±0.01 <sup>d</sup>
7	35	50	60	28.32±2.90 <sup>a</sup>	67.02±3.60 <sup>b</sup>	2.90±0.03 <sup>bcd</sup>
8	15	65	30	45.76±1.06 <sup>d</sup>	66.81±1.64 <sup>b</sup>	3.20±0.02 <sup>d</sup>
9	25	65	60	45.68±2.37 <sup>d</sup>	64.40±2.81 <sup>ab</sup>	2.64±0.05 <sup>ab</sup>
10	35	65	30	28.85±1.85 <sup>a</sup>	66.02±1.79 <sup>b</sup>	3.01±0.07 <sup>bcd</sup>
11	25	65	60	44.13±2.56 <sup>d</sup>	63.10±2.72 <sup>ab</sup>	2.76±0.07 <sup>abcd</sup>
12	15	65	90	63.51±1.54 <sup>e</sup>	76.55±0.70 <sup>c</sup>	3.96±0.09 <sup>e</sup>
13	35	65	90	46.41±1.57 <sup>d</sup>	66.67±1.52 <sup>b</sup>	3.16±0.06 <sup>cd</sup>
14	25	65	60	45.35±2.34 <sup>d</sup>	62.97±2.07 <sup>ab</sup>	2.93±0.05 <sup>abcd</sup>
15	25	80	30	36.87±1.13 <sup>bc</sup>	66.14±2.01 <sup>b</sup>	2.87±0.06 <sup>bcd</sup>
16	15	80	60	28.07±3.26 <sup>a</sup>	57.67±3.81 <sup>a</sup>	2.84±0.01 <sup>bcd</sup>
17	25	80	90	32.06±1.38 <sup>ab</sup>	67.45±1.98 <sup>b</sup>	2.27±0.02 <sup>a</sup>

\*Mean±S.E. with different superscripts row wise differ significantly (P<0.05)

**Table 6: Antioxidant activities of aqueous aloe vera extract (Mean±SE) \***

Sl.No.	Time (min.)	Temp. (°C)	Conc. (%)	DPPH(% Inhibition)	ABTS(% Inhibition)	Total Phenolics (GA Equivalent mg/g)
1	25	65	99	38.71±3.36 <sup>abcd</sup>	35.53±1.14 <sup>a</sup>	2.59±0.25 <sup>ab</sup>
2	25	65	99	41.92±3.67 <sup>abcd</sup>	54.63±1.76 <sup>efg</sup>	3.01±0.13 <sup>ab</sup>
3	25	43.78	99	42.61±1.43 <sup>bcd</sup>	54.23±1.62 <sup>efg</sup>	2.93±0.29 <sup>ab</sup>
4	25	65	99	41.92±3.67 <sup>abcd</sup>	54.38±2.37 <sup>efg</sup>	3.20±0.12 <sup>bc</sup>
5	39.14	65	99	33.49±1.11 <sup>ab</sup>	55.47±1.90 <sup>fg</sup>	2.82±0.29 <sup>ab</sup>
6	25	65	99	41.92±3.34 <sup>abcd</sup>	47.27±3.11 <sup>dce</sup>	2.64±0.35 <sup>ab</sup>
7	35	80	99	42.53±1.22 <sup>bcd</sup>	55.13±2.10 <sup>fg</sup>	2.39±0.25 <sup>a</sup>
8	35	80	99	32.56±0.97 <sup>a</sup>	42.53±1.22 <sup>abcd</sup>	2.66±0.28 <sup>ab</sup>
9	15	80	99	48.09±3.62 <sup>d</sup>	59.38±2.49 <sup>g</sup>	3.72±0.24 <sup>c</sup>
10	10.85	65	99	43.72±3.24 <sup>cd</sup>	49.62±3.11 <sup>def</sup>	2.93±0.21 <sup>ab</sup>
11	25	65	99	41.92±3.36 <sup>abcd</sup>	36.87±3.60 <sup>ab</sup>	2.64±0.17 <sup>ab</sup>
12	15	50	99	42.20±3.55 <sup>abcd</sup>	41.92±3.67 <sup>abc</sup>	2.61±0.11 <sup>ab</sup>
13	25	86.21	99	38.22±1.99 <sup>abc</sup>	43.20±2.85 <sup>bcd</sup>	2.27±0.17 <sup>a</sup>

\*Mean±S.E. with different superscripts differ significantly (P<0.05)

for 15 min. and lowest for extract obtained by 30 % ethanol at 65°C for 25 min. and 60% ethanol solvent for 80°C for 15 min. as well as 50°C for 35 min. The lower antioxidant efficiency at higher temperature might be due to depletion

of active ingredients upon exposure to higher temperature for longer duration. A linear correlation between radical scavenging activity and poly-phenolic content has been reported in an extensive range of extracts of vegetables and fruits (Robards *et al.*, 1999; Kim *et al.*, 2013).

The *in-vitro* DPPH (% inhibition) of aqueous extract of *aloe vera* was recorded highest for the combination of 99% concentration at 80°C temperature for 15 min. amongst all 13 runs, however, under prolonged exposure at same temperature and concentration resulted in poor DPPH value. The lowest value was observed for extract obtained for 35 min. at 80°C. The ABTS (% inhibition) and total phenolics also followed similar trends and recorded highest value for 99% aqueous concentration at 80°C for 35 min. The total phenolics value obtained during aqueous extraction was lower than their alcoholic extracts. This could be due to better extraction of phenolic compounds in ethanol medium. The high scavenging activity might be due to their respective high phenolic contents. Gorinstein *et al.* (2003) reported positive correlation between phenolic content and antioxidant potential of compounds. However, better free radical scavenging efficiency was noticed in extract extracted in alcoholic (ethanol) medium.

#### **Antioxidant efficacy of ethanolic and aqueous extracts of cinnamon bark**

The results for antioxidant activity of aqueous and ethanolic extract of cinnamon bark obtained by various combinations of extraction time (3, 6, 9 h), extraction temperature (40°C, 60°C and 80°C) in ethanol concentration (70%, 80% and 90%) and aqueous concentration (99%) are presented in

Table 7 and Table 8, respectively. A total of 17 and 13 different types of extracts were obtained having various combinations of extraction time, temperature and solvent concentration.

Cinnamon bark extract exhibited significant radical scavenging activity and the scavenging activity increased with the increase in concentration of organic solvent (Mathew and Abraham, 2006). In present study, it exhibited good scavenging activity at all concentration studied. The *in-vitro* antioxidant activity of alcoholic extract of cinnamon bark as observed by radical scavenging activity (DPPH and ABTS) and total phenolics, was recorded highest ( $P<0.05$ ) for a combination of 90% ethanol concentration at 60°C temperature for 9h and lowest for extract obtained by 70% ethanol at 80°C for 6h as well as 80% ethanol solvent for 40°C for 3h. The lower antioxidant efficiency at higher temperature might be due to depletion of active ingredient upon exposure to higher temperature for longer duration. A linear correlation between radical scavenging activity and poly-phenolic content has been reported in an extensive range of extracts of vegetables and fruits (Robards *et al.*, 1999; Kim *et al.*, 2013).

The *in-vitro* DPPH (% inhibition) of aqueous extract of cinnamon bark was recorded highest for the combination of 99% concentration at 80°C temperature for 3h amongst

**Table 7: Antioxidant activities of ethanolic cinnamon extract (Mean $\pm$ SE) \***

Sl. No.	Time (h)	Temp. (°C)	Conc. (%)	DPPH (% Inhibition)	ABTS (% Inhibition)	Total Phenolics (GA Equivalent mg/g)
1	3	60	70	32.48 $\pm$ 3.67 <sup>ab</sup>	74.86 $\pm$ 1.12 <sup>def</sup>	3.03 $\pm$ 0.19 <sup>bcd</sup>
2	6	80	70	27.54 $\pm$ 2.84 <sup>a</sup>	75.79 $\pm$ 1.94 <sup>e</sup>	2.94 $\pm$ 0.35 <sup>bcd</sup>
3	3	40	80	26.90 $\pm$ 2.73 <sup>a</sup>	60.73 $\pm$ 1.34 <sup>abc</sup>	3.02 $\pm$ 0.28 <sup>abcd</sup>
4	6	60	80	35.77 $\pm$ 1.71 <sup>ab</sup>	69.10 $\pm$ 1.60 <sup>bcd</sup>	3.42 $\pm$ 0.05 <sup>cde</sup>
5	9	80	80	33.38 $\pm$ 3.32 <sup>ab</sup>	62.18 $\pm$ 1.99 <sup>ab</sup>	3.09 $\pm$ 0.37 <sup>bcd</sup>
6	6	60	80	33.05 $\pm$ 2.74 <sup>ab</sup>	68.65 $\pm$ 4.1 <sup>bcd</sup>	3.37 $\pm$ 0.17 <sup>cde</sup>
7	6	80	90	32.79 $\pm$ 3.61 <sup>ab</sup>	56.26 $\pm$ 2.24 <sup>a</sup>	2.00 $\pm$ 0.13 <sup>a</sup>
8	6	40	90	30.27 $\pm$ 2.28 <sup>ab</sup>	58.07 $\pm$ 2.88 <sup>a</sup>	3.04 $\pm$ 0.21 <sup>cde</sup>
9	6	60	80	33.54 $\pm$ 3.42 <sup>ab</sup>	69.38 $\pm$ 1.74 <sup>cde</sup>	3.36 $\pm$ 0.14 <sup>cde</sup>
10	3	60	90	29.89 $\pm$ 3.13 <sup>ab</sup>	67.98 $\pm$ 1.79 <sup>bcd</sup>	2.93 $\pm$ 0.43 <sup>bcd</sup>
11	6	40	70	35.50 $\pm$ 3.94 <sup>ab</sup>	59.21 $\pm$ 3.02 <sup>a</sup>	2.69 $\pm$ 0.30 <sup>abcd</sup>
12	3	80	80	27.40 $\pm$ 2.15 <sup>ab</sup>	62.81 $\pm$ 1.05 <sup>abc</sup>	2.56 $\pm$ 0.45 <sup>abc</sup>
13	6	60	80	35.06 $\pm$ 2.35 <sup>ab</sup>	70.99 $\pm$ 1.23 <sup>de</sup>	3.47 $\pm$ 0.22 <sup>de</sup>
14	6	60	80	35.44 $\pm$ 4.01 <sup>ab</sup>	71.76 $\pm$ 1.54 <sup>de</sup>	3.27 $\pm$ 0.25 <sup>cde</sup>
15	9	60	70	35.06 $\pm$ 3.51 <sup>b</sup>	62.89 $\pm$ 2.15 <sup>abc</sup>	2.72 $\pm$ 0.19 <sup>abcd</sup>
16	9	60	90	64.61 $\pm$ 3.72 <sup>c</sup>	83.58 $\pm$ 1.83 <sup>d</sup>	3.85 $\pm$ 0.14 <sup>e</sup>
17	9	40	80	32.04 $\pm$ 3.60 <sup>ab</sup>	75.90 $\pm$ 3.06 <sup>e</sup>	2.25 $\pm$ 0.16 <sup>ab</sup>

\*Mean $\pm$ S.E. with different superscripts differ significantly ( $P<0.05$ )

**Table 8: Antioxidant activities of aqueous cinnamon extract (Mean±SE) \***

Sl. No.	Time (h)	Temp. (°C)	Conc. (%)	DPPH (% Inhibition)	ABTS (% Inhibition)	Total Phenolics (GA Equivalent mg/g)
1	9	40	99	15.85±3.22 <sup>ab</sup>	30.63±2.16 <sup>bc</sup>	2.56±0.22 <sup>bcd</sup>
2	10.24	60	99	24.13±1.15 <sup>bcd</sup>	28.83±2.03 <sup>bc</sup>	2.37±0.29 <sup>abcd</sup>
3	6	60	99	25.61±1.23 <sup>cd</sup>	30.36±1.42 <sup>bc</sup>	2.58±0.25 <sup>cd</sup>
4	1.75	60	99	29.43±1.92 <sup>d</sup>	34.25±1.07 <sup>cd</sup>	2.37±0.31 <sup>abcd</sup>
5	6	31.71	99	28.78±4.29 <sup>cd</sup>	40.67±2.99 <sup>def</sup>	1.81±0.13 <sup>a</sup>
6	3	40	99	17.89±2.70 <sup>abc</sup>	27.05±2.49 <sup>b</sup>	2.75±0.14 <sup>cd</sup>
7	6	60	99	28.41±1.97 <sup>d</sup>	19.17±1.41 <sup>a</sup>	2.87±0.21 <sup>cd</sup>
8	9	80	99	22.01±1.92 <sup>abcd</sup>	40.57±3.04 <sup>def</sup>	2.39±0.19 <sup>abcd</sup>
9	6	88.82	99	27.79±2.76 <sup>d</sup>	41.48±1.26 <sup>def</sup>	1.90±0.16 <sup>ab</sup>
10	6	60	99	17.28±2.76 <sup>ab</sup>	27.60±1.18 <sup>b</sup>	2.64±0.19 <sup>cd</sup>
11	6	60	99	21.16±3.21 <sup>abcd</sup>	40.65±3.16 <sup>def</sup>	2.20±0.18 <sup>abcd</sup>
12	3	80	99	29.43±1.98 <sup>e</sup>	46.17±1.14 <sup>f</sup>	2.76±0.10 <sup>d</sup>
13	6	60	99	15.53±1.57 <sup>a</sup>	36.93±2.08 <sup>de</sup>	2.10±0.17 <sup>abc</sup>

\*Mean±S.E. with different superscripts differ significantly ( $P<0.05$ )

all 13 runs. The lowest value was observed for extract obtained for 6h at 60°C. The ABTS (% inhibition) and total phenolics also followed similar trends and recorded highest value for 99% aqueous concentration at 80°C for 3h. The total phenolics value obtained during aqueous extraction was lower than their alcoholic extracts. Various findings such as Chan *et al.* (2012) reported higher content of phenolics and flavonoids in cinnamon bark aqueous extract and correlated their potent antioxidant effect in model meat system.

Cinnamon bark contains an essential oil containing cinnamaldehyde and eugenol and these compounds are mostly responsible for positive health benefits to consumers. Cinnamaldehyde being an organic compound dissolves in water very poorly and it is very soluble in alcohol and ether. Thus the aqueous extract of cinnamon bark showed comparatively lower antioxidant activity than ethanolic extract. Mathew and Abraham (2006) reported higher antioxidant compounds in alcoholic extract of cinnamon bark, capable to effectively scavenge reactive oxygen species including superoxide and hydroxyl radicals under *in-vitro* experimental conditions.

## CONCLUSION

The results indicated that selective extraction from

natural sources, by an appropriate solvent, is important for obtaining fractions with high antioxidant and high antibacterial activity. Among all the solvents (aqueous and alcoholic) used in the present study, 90% ethanol were found to be the solvent of choice for extraction of active compounds from *aloe vera* and cinnamon bark in order to get maximum antioxidant activity as well as higher total phenolic content. Extracts with highest free radical scavenging activity were recorded in *aloe vera* and cinnamon bark extracted in 90% ethanol at 65°C temperature for 15 min. and 60°C temperature for 9h.

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## REFERENCES

- Alves, D.S., Pérez-Fons, L., Estepa, A. and Micol, V. 2004. Membrane-related effects underlying the biological activity of the anthraquinonesemodin and barbaloin. *Biochem. Pharmacol.*, **68**: 549-61.

- Chan, K.W., Khong, N.M.H., Iqbal, S. and Ismail, M. 2012. Simulated gastrointestinal pH condition improves antioxidant properties of wheat and rice flours. *Int. J. Mol. Sci.*, **13**: 7496-507.
- Chen, C.W. and Ho, C.T. 1995. Antioxidant properties of polyphenols extracted from green and black teas. *J. Food Lipid*, **2**: 35-46.
- Contini, M., Baccelloni, S., Massantini, R. and Anelli, G. 2008. Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. *Food Chem.*, **110**: 659-669.
- Gorinstein, S., Yamamoto, K., Katrich, E., Leontowicz, H., Lojek, A., Leontowicz, M., Ciz, M., Goshev, I., Shalev, U. and Trakhtenberg, S. 2003. Antioxidative properties of Jaffa sweeties and grapefruits and their influence on lipid metabolism and plasma antioxidative potential in rats. *J. Biosci. Biotechnol. Biochem.*, **67**: 907-10.
- Habeeb, F., Shakir, E., Bradbury, F., Cameron, P., Taravati, M.R., Drummond, A. J., Gray, A.I. and Ferro, V.A. 2007. Screening methods used to determine the anti-microbial properties of *aloe vera* inner gel. *Methods.*, **42**: 315-20.
- Hamman J.H. 2008. Composition and applications of *aloe vera* leaf gel. *Molecules*, **13**: 1599-16.
- Harris, J.C., Cottrell, S.L., Plunmer, S. and Llyod, D. 2001. Antimicrobial properties of *Allium sativum* (garlic). *Appl. Microbiol. Biotechnol.*, **57**: 282- 86.
- Jayaprakash, G.K., Rao, L.J. and Sakariah, K.K. 1997. Chemical composition of the volatiles from oil from the fruits of *Cinnamomum zeylanicum* Blume. *Flavour Fragrance J.*, **12**: 331.
- Kato, K., Terao, S., Shimamoto, N. and Hirata, M. 1988. Studies on scavengers of active oxygen species. 1. Synthesis and biological activity of 2-O-alkylascorbic acids. *J. Medicinal Chem.*, **31**: 793-98.
- Kim, S.J., Cho, A.R. and Han, J. 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, **29**: 112-20.
- Mathew, S. and Abraham, T.E. 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chem.*, **94**: 520-528.
- Raccach, M. 1984. The antimicrobial activity of phenolic antioxidants in foods: a review. *J. Food Safety*, **6**: 141-70.
- Raina, V.K., Srivastava, S.K., Aggarwal, K.K., Ramesh, S. and Kumar, S. 2001. Essential oil composition of *Cinnamomum zeylanicum* leaves from Little Andaman, India. *Flavour Fragrance J.*, **16**: 374-76.
- Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. and Glover, W. 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.*, **66**: 401-36.
- Shah, M.A., Bosco, S.J.D and Mir, S.A. 2014. Plant extracts as natural antioxidants in meat and meat products. *Meat Sci.*, **98**: 21-33.
- Shirwaikar, A., Rajendran, K. and Punitha, I.S.R. 2006. *In-vitro* antioxidant studies on the benzyl tetra-quinoline alkaloid berberine. *Biol. Pharma. Bull.*, **29**: 1906-10.
- Simic, A., Sokovic, M.D., Ristic, M., Grujic-Jovanovic, S., Vukojevic, J. and Marin, P.D. 2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. *Phytother. Res.*, **18**: 713-717.
- Thoo, Y.Y., Ho, S.K., Liang, J.Y., Ho, C.W., Tan, C.P. 2010. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chem.*, **120**: 290-295.
- Yuan, Y.V., Bone, D.E. and Carrington M.F. 2005. Antioxidant activity of dulse (*Palmariapalmata*) extract evaluated *in-vitro*. *Food Chem.*, **91**: 485-94.