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# Evaluation of Mineral Contents and Antioxidant Ability of Black Pepper Seed Oil by Soxhlet and Cold Pressing

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## ABSTRACT

In the present study, the evaluation of mineral contents of black pepper seeds is determined by atomic absorption spectrometry. The mineral analysis of the Atomic Absorption Spectrometry (AAS) of black pepper consist of Manganese (Mn) 2.282 ± 0.01 µg/ml, Magnesium (Mg) 8.148 ± 0.08 µg/ml, Copper (Cu) 0.27 ± 0.005 µg/ml, Zinc (Zn) 0.593 ± 0.01 µg/ml and Iron (Fe) 0.89 ± 0.005 µg/ml. The efficiency and stability of natural antioxidants in black pepper seed oil are determined by reducing power (FRAP). Free radicals present in oils and fats are responsible for harmful for the body cell. The stability of natural antioxidants of black pepper seed oil and their significance at different concentrations is calculated by One Way ANOVA Test. In the present study, the result showed that the increased concentration of black pepper seed oil by solvent soxhlet extraction and cold pressing (25 µg/ml - 100 µg/ml) has a positive correlation with reducing power assay. The result showed that black pepper oil has ability to remove toxic compounds produced in the body and help maintain the body cells. In addition, the extraction methods can be helpful for the improvement in the preservation of important nutrients that may be sensitive to thermal heat. Cold pressing is a useful method as it can exclude the use of heat as well as organic solvent in soxhlet method.

**Keywords:** Mineral contents (AAS), Reducing assay (FRAP), and One-way ANOVA Test.

## INTRODUCTION:

Black pepper (*Piper nigrum* L.) is commonly used spies in all over word. In the state of dry and ground beans are used in cooking purpose for flavoring and enhance the shell life of the food product. Black pepper is also used in natural medicine, due to its therapeutic properties (Nair, 2011). To enhance the quality control of the spies are essential for consumer safety and evaluating the potentially toxic contaminants such as metal elements (Darko *et al.,* 2014). Trace elements may be incorporated in them due to the processing through drying, grinding, packing and transport step. The number of studies has been reported in determination of metal elements present in plant samples such as spies, herbs and medicinal plants including black pepper (Solak *et al.,* 2012)

Vegetable oils are used as raw material for the various products. During the storage, processing and high temperature exposure is responsible to produced toxic products like peroxides, dimmers, etc, which changes the taste and nutritional quality of food (David & Choe, 2003). Some free radicals present in the body is

causing agent of cancer and first it attack on cell DNA, mutational changes occurred in DNA which finally induced cancer (Ahmad *et al*., 2010; Abdullahi *et al*., 2011). Now a day's scientists are interested on natural antioxidant to cure cellular degradation (Ahmad *et al*., 2011; Obinna *et al*., 2009). The focused on natural antioxidant present in plants have been increased and it is consider as non-toxic and environmental friendly (Ahmad *et al*., 2011; Ahmad *et al*., 2010). In recently the plants are consumed as food additives due to their antioxidant activity (Ahmad *et al*., 2011; Abdou, 2011; Sharif *et al*., 2019; Rahman *et al*., 2020).

The regenerative tissues of Black pepper have antioxidant activities such as in-vitro shoots, callus, roots and in-vitro plantlets due to presence of flavanoids and phenolic contents (Ahmad *et al*., 2010). The effect of black pepper on membrane lipid peroxidation has been checked on rats both enzymetic and non-enzymatic antioxidant activities and found that black pepper reduced the oxidative stress (Vijayakumar *et al*., 2004). Black pepper also prevents the intestine induced lipid peroxidation, oxidative stress, lung carcinogenesis, different radicals such as hydroxyl and super oxides radicals and human lipoxygenase (Neha and Mishra, 2011;Vijayakumar *et al*., 2004; Muhtaseb *et al*., 2008; Naseri and Yahyavi, 2007; Selvendiran and Sakthisekaran, 2004).

The present study is conducted with the following aims and objectives

a) To evaluate mineral contents present in black pepper seed.
b) To determine the antioxidant activity of black pepper seeds oil by solvent soxhlet and cold pressing
c) To find out the significance and correlation of antioxidant activity of black pepper seed oil by two different extraction methods i.e solvent soxhlet extraction and cold pressing through One Way ANOVA Test.

## MATERIALS AND METHODS:

### Sample Collection and Preparation

Black pepper seeds were collected from local market, Rawalpindi. These seeds were washed through water and dried under shade. After that the sample was grounded in powered form with the help of grinder. The sample was stored in well plastic bags and placed in refrigerator at 4 $^{o}$C for further examination.

### Extraction of black pepper oil through Soxhlet apparatus

The black pepper sample was weighted and 15 grams of the sample was set in the filter paper thimble. The sample was loaded in the loading chamber and extraction can be done for 6 to 8 hours in soxhlet with 120mL of n-hexane which was present in the conical flask. The oil was changed into the solvent and changing in its color was noted (Quan *et al.,* 2004). When extraction had completed, the n-hexane (solvent) was allowed to escape by using vacuum evaporator at 45$^{o}$C. The oil was placed at room temperature for the evaporation of remaining solvent from the sample for 24 hours. The process was done for three times in the sake of accuracy and black pepper oil was saved at 4$^{o}$C for further analysis.

### Extraction of black pepper seed oil through Cold pressing

Black pepper seeds were pressed at room temperature by cold pressing machine without any thermal treatment. The seeds oil was collected in glass bottle for further analysis.

### Mineral analysis (AAS)

The black pepper seed sample was subjected to mineral analysis by using Atomic Absorption Spectro-photometer (AAS) were following (Lindsay and Norvell, 1978) to evaluate the concentration of ele-ments present in black pepper seeds. The mineral under study were Manganese (Mn), Magnesium (Mg), Copper (Cu), Zinc (Zn) and Iron (Fe). This technique is mostly used to determine the concentration of a particular metal element within a sample.

### Reducing power assay (FRAP)

Antioxidant properties of black pepper seed oil was determined by reducing power assay (FRAP). This assay was described by Chung *et al*. (2005). In this assay different dilutions were prepared from black pepper seed. 2 ml of each dilution was taken in the test tubes, added 2ml of 0.2M of phosphate buffer (pH 6.6) and 2ml of potassium ferricynide (1mg/ml). Incubated it at 50 $^{o}$C for 20 minutes and then cool it. Added 2 ml of tris acetic acid (1%) then the solvent was centrifuged at 3000rpm for 10 minutes. The super-

natant of 0.25 ml was mixed with 0.25 ml dist. water and 0.1% of $FeCl_3$ (0.5 ml) and left it for 10 minutes. Measured its absorbance was at 700nm. Increased absorbance of reaction mixture showed the higher antioxidant activity.

### Statistical analysis

Each parameter was analyzed with the help of average value and standard deviation (S.D). The One Way ANOVA Test is performed to determine the significance and correlation of antioxidant activity of black pepper seed oil by solvent soxhlet extraction and cold pressing

## RESULTS AND DISCUSSION:

In present study, the mineral analysis of the atomic absorption spectrometry of black pepper is consist of Manganese (Mn) 2.282 ± 0.01 µg/ml, Magnesium (Mg) 8.148 ± 0.08 µg/ml, Copper (Cu) 0.27 ± 0.005 µg/ml, Zinc (Zn) 0.593 ± 0.01 µg/ml and Iron (Fe) 0.89 ± 0.005 µg/ml as shown in **(Table 1)**.

**Table 1:** Minerals contents present in Black pepper seed (µg/ml).

| Mineral analysis | Black pepper Result (µg/ml) | Quantity (%) |
|---|---|---|
| **Mn** | 2.282 ± 0.01 | 19 |
| **Mg** | 8.148 ± 0.08 | 67 |
| **Cu** | 0.27 ± 0.005 | 2 |
| **Zn** | 0.593 ± 0.01 | 5 |
| **Fe** | 0.89 ± 0.005 | 7 |

Means (n = 3) ± S.D

It is reported that the bouillon cubes contain low levels of iron and zinc reported by (Akpanyung, 2005) which is used in food preparations, which is positively correlated that the low level of iron and zinc present in black pepper seed oil. It is utilized as food additives and improves the shell life of food product reported by (Krause, 2019). Comparatively lower concentrations of Mg, Fe, Mn, Zn and Cu were reported in **(Table 2)** by (Lee *et al*., 2020; Al-Jasass and Al-Jasser 2012: Shahen *et al*., 2019; Ozcan and Akbulut 2007).

**Table 2:** Comparisons of Mineral contents present in Black pepper seed oil with reported mineral contents of black pepper pericarp.

| Mineral contents | This Study (Black pepper seed oil) | Black pepper pericarp Lee *et al*., (2020) | Black pepper pericarp Al-Jasass and Al-Jasser, (2012) | Black pepper pericarp Ozcan and Akbulut, (2007) |
|---|---|---|---|---|
| | (µg/ml) | (mg/100g) | (mg/100g) | (mg/100g) |
| **Mn** | 2.282 ± 0.01 | 24.52 ± 2.80 | 3.5 ± 0.2 | 12.97 ± 2.28 |
| **Mg** | 8.148 ± 0.08 | 209.97 ± 0.61 | 52.0 ± 8.0 | 148.69 ± 21.58 |
| **Cu** | 0.27 ± 0.005 | 0.90 ± 0.02 | 1.3 ± 0.1 | 0.48 ± 0.06 |
| **Zn** | 0.593 ± 0.01 | 1.02 ± 0.01 | 0.9 ± 0.1 | 0.93 ± 0.09 |
| **Fe** | 0.89 ± 0.005 | 42.44 ± 1.08 | 20.5 ± 0.5 | 8.92 ± 1.14 |

Means (n = 3) ± S.D

The Mg level in black pepper was more than that mentioned in spinach (87 mg/100 g), tuna (64 mg/100 g) and brown rice (44 mg/100 g), and only slightly lower than that in almond (270 mg/100 g) had reported by (Jones, 2017). Mg is important minerals in forming bone, strengthening heart functions, relaxing muscle, conducting memory and metabolizing glucose was reported by (Veronese *et al.*, 2016). Thus black pepper seed oil is showed stronger health beneficial effects on human beings. (Bouba *et al*., 2012) had studied on the nutritive analysis, mineral analysis and vitamin analysis of twenty commonly used wild plants as spice in Cameroon. They found that all the spices have less moisture contents ranging from 7.7 g/100g to 10.5 g/100g and greater ash value ranging from 7.7 g/100g to 10.5 g/100g. The lipid content was comparatively higher found in *Monodora myristica* as 53.4 g/100g, *Xylopia aethiopica* as 33.7 g/100g, *Fagarale prieuri* as 32.1 g/100g and *Aframomumda niellii* as 23.1 g/100g. Their studies indicate that spies are good source (Sielicka & Samotyja, 2013) were studied on the antioxidant properties of extracts obtained from cold-

pressed oils using different solvents. They were used five solvents which were acetone, methanol, acetone: water (50:50 V/V), methanol: water (50:50 V/V) and methanol: water (70:30 V/V). They mainly focused in the determination of antiradical properties by using the antioxidant assay (ferric reducing antioxidant power). They observed that antioxidant activities of samples were different in different solvents. This study showed that different methods of extraction are responsible for showing difference in reading of the samples. (Oliver *et al*., 2006) worked on chemical and antioxidant abilities of twelve essential oils of spices. Antioxidant efficiency was determined by four different assays such as oxidative stability of fat assay (RANCIMAT), DPPH assay, ferric reducing antioxidant power assay (FRAP) and thio-barbituric acid reactive species assay (TBARS). The antioxidant activities of different oils were arranged on bases of increased antioxidant power by descending order which as followed *Syzygium aromaticum, Ocimum basilicum*, *Laurusnobilis*, *Coriandrum sativum*, *Myristica fragrans, Piper nigrum*, *Helichrysum italicum, Mentha piperita*, *Marjorana hortensis*, *Cinnamomum zeylanicum*, *Salvia officinalis* and *Foeniculum vulgare* respectively. The black pepper oil was used as insect-repellent, insecticidal and inhibition in developmental activities of an important wheat grain pest *Tribolium castaneum* and it was observed that larval stage and adult appearance were reduced with increased dose of black pepper oil had reported by (Upahyay & Jaiswal, 2007). (Uyoh *et al*., 2013) were worked on two frequently common used spices in Nigeria which were *Piper nigrum* and *Monodora myristica* and their seeds and leaf were utilized as natural antioxidants *in vitro* assay systems. On the comparisons with black pepper extract, the *Monodora myristica* extract was better in antioxidant ability and all the extracts depend on their concentration for antioxidant activities. This work showed that's spices plant has ability to utilize as natural antioxidant and would help to reduce the free radical damages when consumed. (Zhang & Xu, 2015) had studied on the antioxidant abilities of black pepper and white pepper oils. Their antioxidant activities were dependent on concentration of oil. White black pepper oil has high antioxidant activity as compared to black pepper oil. These results indicated that white pepper and black pepper essential oil used as a natural antioxidant. In present study it is indicated that reducing power of black pepper seed oil and ascorbic acid has direct relationship to the concentration. Absorbance of black pepper seeds oil obtained through soxhlet and ascorbic acid at different concentrations (25 µg/ml - 100 µg/ml) is shown in **(Fig. 1A)**.

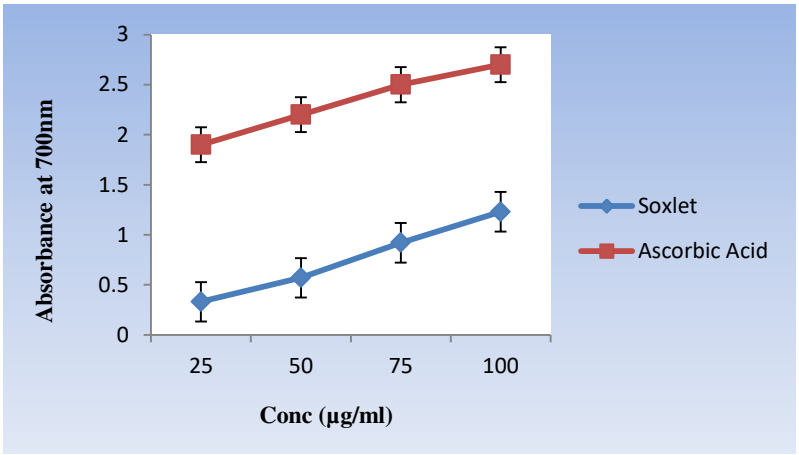


**Fig. 1A:** Reducing power ability of black pepper seeds oil through soxlet and ascorbic acid.

Absorbance of black pepper seeds oil obtained through cold pressing and ascorbic acid at different concentrations (25 µg/ml - 100 µg/ml) is shown in **(Fig. 1B)**. Comparison of absorbance of black pepper seeds oil extracted from soxhlet and cold pressing at different concentrations (25 µg/ml - 100 µg/ml) is shown in **(Fig. 1C)**. It is cleared from the figures that absorbance increased at higher concentrations which showed that the higher antioxidant activity at higher concentration. The reducing power ability of the black pepper seeds oil had positive correlation with the reported reducing power ability of black pepper oil

(Zhang and Xu, 2015). The significance of antioxidant activity of black pepper seed oil by soxhlet and cold pressing at different concentrations (25 µg/ml - 100 µg/ml) is determined by one way ANOVA Test.

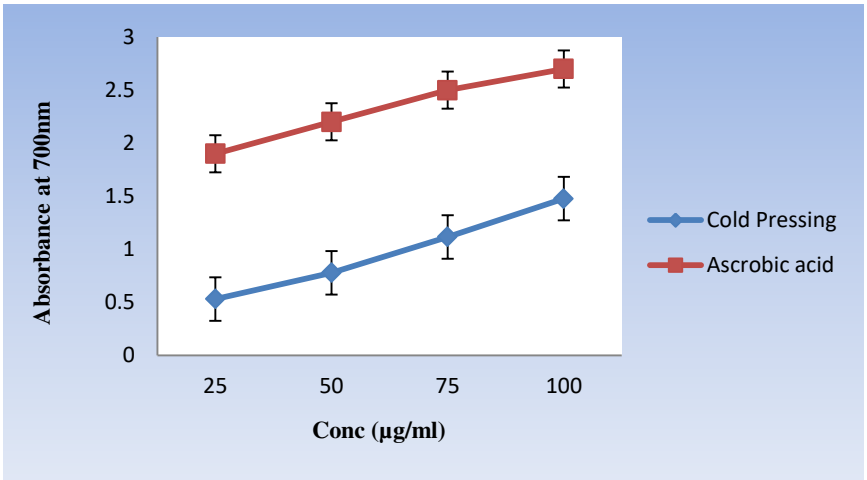


**Fig. 1B:** Reducing power ability of black pepper seeds oil through cold pressing and ascorbic acid.

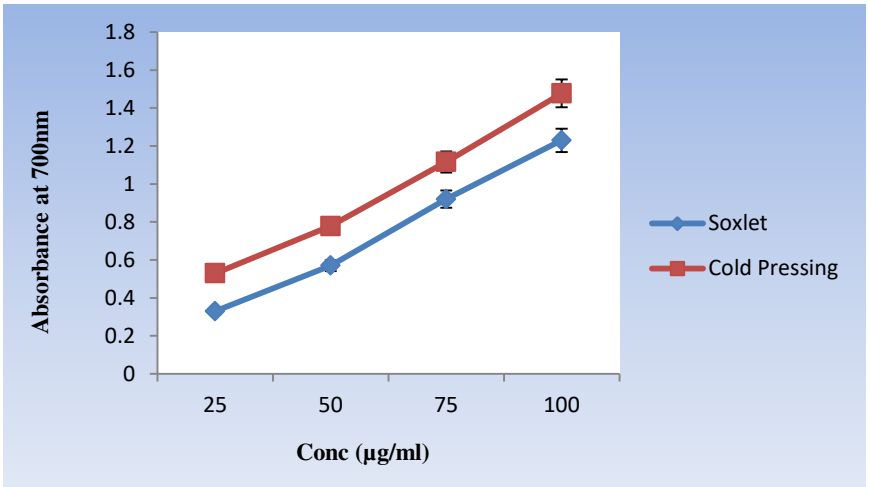


**Fig. 1C:** Comparison of reducing power ability of black pepper seeds oil through soxhlet and cold pressing.

For the sake of accuracy data were recorded as mean ± S.D, the statically significant value of sample is less than 0.05 ($P < 0.05$) while the P value of sample is greater than ($P < 0.05$) than there is not significant correction present between two extraction methods. The value of Probability value of antioxidant activity of black pepper seed oil by soxhlet and cold pressing at different concentrations (25 µg/ml - 100 µg/ml) is determined by One Way ANOVA Test as reported in (**Table 3**), to evaluate the efficiency and stability of black pepper seed oil extracted by soxhlet and cold pressing.

**Table 3:** Determination of significance of black pepper seed oil by soxhlet and cold pressing.

| Sr. No | Concentration of oil sample (µg/ml) | Control (Ascorbic Acid) (µg/ml) | Black pepper seed oil | | P value |
|---|---|---|---|---|---|
| | | | Solvent soxhlet (µg/ml) | Cold pressing (µg/ml) | One Way ANOVA Test |
| **1** | 25 | 1.9 ± 0.01 | 0.33 ± 0.005 | 0.53 ± 0.005 | 0.000 < 0.05 |
| **2** | 50 | 2.2 ± 0.01 | 0.57 ± 0.01 | 0.77 ± 0.005 | 0.000 < 0.05 |
| **3** | 75 | 2.5 ± 0.01 | 0.92 ± 0.005 | 1.11 ± 0.005 | 0.000 < 0.05 |
| **4** | 100 | 2.7± 0.01 | 1.23 ± 0.01 | 1.4 ± 0.005 | 0.000 < 0.05 |

Mean value ± S.D (n=3) Significant difference of black pepper oil between soxhlet and cold pressing ($p < 0.05$)

The significance of black pepper seed oils by soxhlet and cold pressing is P value (0.000) is less than 0.05 then the data is statically more significant. Thus the data is significant and antioxidant property is highly correlated with each other and black pepper seed oil is used as antioxidant. This P value of black pepper oil is positive correlation with vegetable oils had been reported by (Valantina & Neelamegam, 2015). These results suggested that the black pepper seeds oil has good potential of natural antioxidant. It has been utilized as antioxidant in medicines, anti cancer agents and other cosmetic products (Ahmad *et al.,* 2024).

## CONCLUSION:

The outcomes of this study indicate that black pepper seed oil has stability and good efficiency as affected by two different extraction methods. The result also indicates that natural antioxidants present in black pepper seed oils are significant and their anti-radical activity increased rapidly with increased concentration. The black pepper oil is revealed that it has less adverse effect on human health. Black pepper seed oil has important minerals in forming bone, strengthening heart functions, relaxing muscle, conducting memory and metabolizing glucose. Black pepper seed oil is indicated that it has great potential to use as natural antioxidants and as anti-radical agents in various useful products for human health. Further investigation is also recommended to determine the different aspects of black pepper seed oil which are beneficial for mankind.


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## CONFLICTS OF INTEREST:

The authors declare no conflicts of interest

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