INT. J. BIOL. BIOTECH., 21 (2): 195-201, 2024.

PHYSICOCHEMICAL PROPERTIES OF BLACK PEPPER (*PIPER NIGRUM* L.) SEED OIL AS AFFECTED BY DIFFERENT EXTRACTION METHODS

Usman Ahmad[1,*], Naveed Iqbal Raja[2], Zia-ur-Rehman Mashwani[2], Rahmatullah Qureshi[2] and Rahmat Wali[2]

[1]*Department of Biology, PMAS Arid Agriculture University Rawalpindi – 46300, Pakistan*
[2]*Department of Botany, PMAS Arid Agriculture University Rawalpindi – 46300, Pakistan*
*Corresponding Author: Email: usmanahmad239@yahoo.com

ABSTRACT

This present study deals with the physicochemical characteristics and some proximate properties of black pepper (*Piper nigrum* L.) seeds oil, was determined by two extractions methods which are solvent based extraction (soxhlet apparatus) and cold pressing. The proximate analysis of black pepper seeds had been determined which was found as moisture content 11.1%, dry matter content 88.9%, ash content 2.5%, protein content 13.13%, fat content 6% and crude fiber content 8.5%. The physicochemical characterization of black pepper seed oil from Soxlet apparatus was saponification value (64.4 mg KOH/g), peroxide (8 meq O_2/g), free fatty acids (3.10 mg/g), acid (6.17 mg/g), specific gravity (0.840 g/cm^3), ester (58.23 mg/g), pH (8.06), iodine (1.27 mg/g) and wax (3.18 mg/g). The physicochemical parameters of black pepper seeds oil from cold pressing was saponification value (109.39 mg KOH/g), peroxide (17 meq O_2/g), free fatty acids (9.87 mg/g), acid (19.63 mg/g), specific gravity (0.849 g/cm^3), ester (89.76 mg/g), pH (8.07), iodine (2.54 mg/g) and wax (4.9 mg/g). The results showed that black pepper seeds oil has high nutritive values which are good source of protein, minerals, fats and dietary fibers. In conclusion, it is used in medicines and to make soaps, skin products, candles and other cosmetics.

Key words: Black Pepper Seeds oil, Proximate, Physicochemical, Soxhlet extraction, Sponification, Rancid

INTRODUCTION

Black pepper is commonly known as flowering vine belonging to family Piperaceae (Nair *et al.,* 2003; Abbasi *et al.,* 2010). *P. nigrum* L. is very important cultivated specie in the piperaceae family, out of 1000 species due to economic values (Bhat *et al*., 1995; Abbasi *et al*., 2010). Pepper is commonly utilized in the curry masalas and major component for preparations of traditional medicines, folk medicine and Ayurveda (Rani *et al.,* 2013).

Piper nigrum L. is very precious due to presence of piperine including its various isomers (Zaveri *et al*., 2010). *P. nigrum* is commonly utilized formation of medicine in India, Latin America and West-Indies for its numerous medicinal values (Scott *et al*., 2008). Black pepper can be utilized as human dietaries, medicine, preservatives and biocontrol agent (Srinivasan, 2007; Awen *et al.,* 2010; Hussain *et al.,* 2011). Black pepper utilized as food for prevention of the chronic ailments due to their physiological effects which is addition to its nutrition values (Sruthi *et al*., 2013). Black pepper is also consumed for its antimicrobial, antioxidant, anticancer, gastro protective effect and anti-inflammatory (Butt *et al*., 2013).

In present scientists from different biological fields try to find out plants which have potential for medicinal uses and biocontrol agents (Hamrapurkar *et al*., 2011). Piperaceae family is good source of medicine and food species (Scott *et al*., 2008). Black pepper is major food spice and two important alkaloids caryophyllene, used for anaethetic activity and nerolidol, used as flavouring agent (Santra *et al*., 2005)

Plants are very important in our daily life and fulfill our needs (Szallasi, 2005). In recent studies free radicals produced in our bodies which are responsible for many diseases (Ahmad *et al*., 2011). Free radicals produced in the body which is responsible for causing oxidation of lipid, reduction of fluidity of membrane, loss of receptor activities, degradation of different enzyme actions and cell inactivation by degradation of proteins present in membranes (Ahmad *et al*., 2011; Kochhar, 2008). Old people and most of herbal practitioners have farm belief that uses of peppercorn powered in green tea prevent asthma (Abbasi *et al*., 2010; Ahmad *et al*., 2010). Local people, herbal practioners and herbal industries used peppercorn for treating diarrhea in all ages (Singh and Duggal, 2009).

Different methods are used to extract oil from plant sources. These are **(1)** Chopping or rendering, **(2)** Cold Pressing or crushing, **(3)** Solvent soxhlet extraction **(4)** Refining. In present study, it is used solvent soxhlet extraction and cold pressing to extract oil from black pepper seeds.

With increased demands of vegetables oil and spice in the country, the investigation has been done on black pepper seeds to find the **(a)** nutritional value of seeds, **(b)** physicochemical composition of seed oil and **(c)** compare the efficiency of two different extraction methods which are extracted by soxhlet apparatus and cold pressing.

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 grinded sample was weighted and 15 grams of the sample was set in the filter paper thimble. The thimble was located in the loading compartment and extracted for about 6 hours in Soxhlet with 120mL of n-hexane which was placed in the 250 mL conical flask. The oil was transferred into the solvent and it was observed by changing in its colour (Quan *et al.*, 2004).

When extraction had done with the help of Soxhlet, the n-hexane (solvent) was evaporated by using vacuum evaporator at 45^{o}C. The oil was permitted to stand at room temperature for the evaporation of remaining n-hexane from the sample for 24 hrs.

The sample was measured to find out the amount of black pepper oil from 15 g of black pepper seeds. 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.

PROXIMATE PARAMETERS

Black pepper powdered sample had analyzed for proximate parameters such as moisture content, dry matter content, ash content, protein content, fat content and crude fiber content with the help of AOAC methods (1990).

PHYSIOCHEMICAL PARAMETERS

Black pepper powdered sample had analyzed for physicochemical parameters such as saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value and iodine value with the help of AOAC methods (2003)

Wax content of the oil was calculated by Gohari *et al*. (2012). 5 grams of black pepper oil was put in a flask and 25 ml of acetone will be added. Then after, solution mixture was settling down and put in freezer for 24 hrs at 4^{o}C to crystal the wax. The acetone represented the insoluble part of the solution which was filtered on pre-weighed filter paper Whatman No.1. Filter paper was dried out in vacuum chamber at 45°C and weighed to obtain the wax contents.

STATISTICAL ANALYSIS

Each parameter was analyzed with the help of average value and standard deviation

RESULTS AND DISCUSSION

PROXIMATE PARAMETERS

The proximate analysis of black pepper seeds had been determined which included moisture content, dry matter content, ash content, protein content, fat content and crude fiber content as shown in table 1. Proximate composition of the black pepper seeds was found as moisture content 11.1%, dry matter content 88.9%, ash content 2.5%, protein content 13.13%, fat content 6% and crude fiber content 8.5%. Plant Foods and seeds are good source of lipid, fibers, proteins, carbohydrates and secondary metabolites. They have major role for improving human health and agriculture (Saleh-E-In and Roy 2007; King, 1970). They are also used for industrial and pharmaceutical purposes to improve the human health (Molwa *et al*., 1990). Lipids mainly consist of fuels, vitamins, flavours, emulsifiers, aromatic compounds and barriers to the environment (Harris, 1989; Nestel, 1990).

The value of protein content (13.13 %) and crude fiber content (8.5 %) of black pepper seed oil correlated with the protein content (11.56 %) and fiber content (16.66 %) of the Bangladesh black pepper seeds (Hossain *et al*.,

2014) due to high protein content it is used in human diet and for fiber content it is good source of dietary fiber. Besides, it is also used to increase feacal bulk and lower gastric cholesterol (Edeoga *et al.*, 2003; Enwere, 1998). Used of such medicinal plants in traditional recipes showed indication that crude fiber is linked by improving sensitivity of insulin which used in the prevention of Type 2 diabetes (Vadivel and Janardhanan, 2005). These medicinal plants played a major role of dietary fiber in human nutrition.

Ash content (2.5 %) of black pepper seeds oil is correlated with the ash content (2.6 %) of kalahari melon (Nyam *et al*., 2009). Ash is the inorganic residues after removing the organic content and moisture from heating, which provides information about the minerals present in the food. Mineral contents are not damaged by heating due to low volatility as compared to other plant stuff. Ash content varies in broad limit for different plants. Ashes give us an idea about of pharmacological effect of plants (Lee, 2005).

Moisture content (13.13 %) of black pepper seeds is linked moisture content (14.91 %) of the *Myristicafragrans* (Thomas and Krishnakumari, 2015). Moisture content linked with the ecological conditions such as temperature, humidity, climate, harvest time and also depend on storage conditions. There is strong relation between moisture contents and fiber which is useful for human health as the fibrous are helpful for digestion and disintegration (Hussain *et al*., 2009).

The fat content (6 %) of black pepper seeds is associated with the fat content of the sea buckthorm (5.81 %) and pomegranate (5.50 %) respectively (Kashif and Ullah, 2013). Fat content are considered as major reservoir of energy and approximately 30 calories used as to keep away from obesity and other associated problems (Harris, 1989). A diet gives energy about 1-2 % as fat which is considered sufficient for humans and excessive consumption is responsible for different cardiovascular diseases such as cancer, aging and atherosclerosis (Antia *et al*., 2006). Black pepper seeds have a good amount of fat, protein, fiber, moisture, and with suitable mineral elements which indicates the positive effect on human diet and showing high nutritive value. The proximate composition black pepper seeds are slightly deviated from Bangladesh black pepper seeds due to environmental conditions and harvesting time. It was found that the black pepper seeds have high nutritive value which was good source of proteins, minerals, fats and dietary fibers.

Table 1. Proximate analysis of black pepper seeds

Proximate Parameters	**Quantity (%)**
Moisture content	11.1 ± 0.77
Dry matter content	88.9 ± 0.77
Ash content	2.5 ± 0.25
Protein content	13.13 ± 0.33
Fat content	6 ± 0.57
Crude fibre content	8.5 ± 0.28

Each value represents Mean ± S.D

Table 2. Physicochemical properties of black pepper seeds oil through Soxlet.

Physicochemical property	**Extraction method** **Soxhlet extraction**
Saponification value (mg KOH/g)	64.4 ± 0.26
Peroxide value (meq O_2/g)	8 ± 0.57
Free fatty Acid (mg/g)	3.10 ± 0.23
Acid value (mg/g)	6.17 ± 0.21
Specific gravity (g)	0.840 ± 0.005
Ester value(mg/g)	58.23 ± 0.16
pH value	8.06± 0.26
Iodine value (mg/g)	1.27 ± 0.15
Wax value(mg/g)	3.18 ± 0.15

Each value represents Mean ± S.D

Table 3 Physicochemical properties of black pepper seeds oil through cold pressing.

Physicochemical property	Extraction method
	Cold pressing
Saponification value (mg KOH/g)	109.39 ± 0.51
Peroxide value (meq O_2/g)	17 ± 0.57
Free fatty Acid (mg/g)	9.87 ± 0.50
Acid value (mg/g)	19.63 ± 0.36
Specific gravity (g/cm^3)	0.849 ± 0.003
Ester value(mg/g)	89.76 ± 0.35
pH value	8.07± 0.19
Iodine value (mg/g)	2.54 ± 0.18
Wax value(mg/g)	4.9 ± 0.15

Each value represents Mean ± S.D

Table 4 Comparison of Physicochemical properties of black pepper seeds oil.

Physicochemical property	Extraction methods	
	Soxhlet extraction	**Cold pressing**
Saponification value (mg KOH/g)	64.4 ± 0.26	109.39 ± 0.51
Peroxide value (meq O_2/g)	8 ± 0.57	17 ± 0.57
Free fatty Acid (mg/g)	3.10 ± 0.23	9.87 ± 0.50
Acid value (mg/g)	6.17 ± 0.21	19.63 ± 0.36
Specific gravity (g/cm^3)	0.840 ± 0.005	0.849 ± 0.003
Ester value(mg/g)	58.23 ± 0.16	89.76 ± 0.35
pH value	8.06± 0.26	8.07± 0.19
Iodine value (mg/g)	1.27 ± 0.15	2.54 ± 0.18
Wax value(mg/g)	3.18 ± 0.15	4.9 ± 0.15

Each value represents Mean ± S.D

PHYSICOCHEMICAL PARAMETERS

The Physico-chemical characterization of black pepper seeds oil had been determined which included saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value, iodine value and wax value. The physicochemical properties of black pepper seeds were carried out through two extraction methods, soxlet apparatus and cold pressing. The physicochemical characterization of black pepper seed oil from soxlet apparatus was saponification value (64.4 mg KOH/g), peroxide (8 meq O_2/g), free fatty acids (3.10 mg/g), acid (6.17 mg/g), specific gravity (0.840 g/cm^3), ester (58.23 mg/g), pH (8.06), iodine (1.27 mg/g) and wax (3.18 mg/g) as shown in table 2.

The physicochemical parameters of black pepper seeds oil from cold pressing was saponification value (109.39 mg KOH/g), peroxide (17 meq O_2/g), free fatty acids (9.87 mg/g), acid (19.63 mg/g), specific gravity (0.849 g/cm^3), ester (89.76 mg/g), pH (8.07), iodine (2.54 mg/g) and wax (4.9 mg/g) shown in table 3.The comparison of black pepper oil through soxlet apparatus and cold pressing was shown in table 4.

The saponification value, peroxide, acid, free fatty acids and iodine of black pepper seeds oil is associated with the saponification value, peroxide, acid and iodine of Bangladesh (Hossain *et al*., 2014) with slight variation. The specific gravity of black pepper seeds oil is linked with specific gravity of Bangladeshi black pepper as well as Indian black pepper seeds oil (Aziz *et al*., 2012). The ester value of the black pepper seeds oil is associated with the Bangladeshi black pepper seed oil (Aziz *et al*., 2012).

Saponification value of oil is determined by refluxing a known amount of a sample with excess of standard alcoholic potassium hydroxide solution and titrating the unused alkali against a standard acid solution. A lower value of sponification number indicates the abundance of high molecular weight fatty acid residues and a higher

value of sponification number indicates the abundance of low molecular weight fatty acid residues. The sponification number is characteristic of particular oil and used for its identification

Saponification value is measurement of checking adulteration. The saponification value was found to be109 mg KOH/g which is less than 177 mg KOH/g reported by Hossain *et al*., (2014) for black pepper oil. High saponification value of 109 mg KOH/g is indication that the oil has potential for uses in the industries.

The pH (8.04) of black pepper oil also indicated that it has nature of alkalinity. It can be used in soap making due to high saponification value (Mir *et al*., 2014). Oils or fats are hydrolyzed by the action of alkali, a mixture of alkali salts of fatty acids and glycerol is formed. Such salts of fatty acids are used in the manufacturing of soap. This hydrolysis using alkali is also called **sponification**.

Peroxide is to determine the oxidation of food stuff for the period of preservation and freshness of the fat content present in oil and is a good indicator of lipid oxidation products. It determined the lipid oxidation which responsible for the presence of saturated fatty acids in it. The elevated level of peroxide oxidation is responsible for the more oxidization of oil**.** High peroxide value of extracted oil predicted that the oil contained lot of free active oxygen species which involved autoxidation of the oil (Mowla*et al*., 1990; Jacobs, 2006). The high peroxide value of balck pepper oil responsible for oxidative rancidity.

Oils or fats, on the exposure of air and light, start giving a foul smell and taste. This phenomenon is called rancidification. We say that the fat or oil has become **rancid**. This is due to attack of oxygen at double bond present in oil produces foul smelling carbonyl compounds. These reasons contribute towards the rancidification.

The iodine value is defined as the number of grams of iodine that combine with 100 g of oil. It gives a measure of unsaturation in oil. Iodine value is determined by the known excess of Wij's solution (iodine monochloride in glacial acetic acid) to a solution of Known weight of the oil. We used Wij's solution because iodine as such does not react with the unsaturated oils. The black pepper oil had low iodine value which also indicated that oil mostly contained unsaturated fatty acids. It is also indicated that unsaturated fatty acids do not allow close packing of the molecules, there will be weaker molecular interactions present in the oils thus responsible for lower melting points. Therefore triglycerides of unsaturated fatty acids of black pepper oil are liquid at room temperature.

Oils and fats are liquid or solids have a greasy touch. The oils are lighter than water (specific gravity of oil is less than 1) thus floating on the surface of water. The high specific gravity of black pepper indicated that it had fatty acids of high molecular weight.

Acid value is to determine the quality of vegetable oils. Acid value defined as1 g of oil required the amount of KOH in mg to neutralize free fatty acids present in it. The free fatty acids content is a usually determined the amount of total fat present in percentage mass-fraction. The high acid value of black pepper seeds oil indicated the higher proportion of the free fatty acids content as compared to other edible oils such as rapeseed oil (0.02 %), soybean oil (0.015 %), sesame seed oil (0.02 %) and palm oil (0.012 %), respectively (Mowla, 1990; Nollet, 2004; Jacobs, 2006). High level of free fatty acid is sign of unfit of oil for utilization purpose. Black pepper oil would not appropriate for edible purposes. It could be refined before utilized for consumption.

It was observed that comparison of physicochemical parameters of compressed seed oil is relatively higher than the Soxhlet apparatus oil. Compressed oil has high acid value which indicates that it is not fit for cooking purpose. It has less oxidative stability due to high peroxide value. That's why cold pressed black pepper oil was more susceptible for oxidation as compared to solvent extracted oil.

CONCLUSION

In the present study, the physicochemical properties of black pepper (*Piper nigrum*) seed oil that show variations between different parameters as affected by using solvent soxhlet extraction and cold pressing. The physicochemical parameters such as saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value and iodine value are used to control the quality of oil for the production of various products as oils are used raw material on industrial scale. It revealed that black pepper seed oil has good source of human dietaries, medicine, preservatives and used to make soaps, skin products, candles and other cosmetics. It also indicates that vegetable oils are non toxic to environment and biodegradable. It also indicates that vegetable oil is used to make biodiesel and used on commercial scale as a substitute for diesel. Further investigations may also be required to determine the different aspects of black pepper seed oil.

REFERENCES

Abbasi, B. H., N. Ahmad, H. Fazal and T. Mahmood (2010). Conventional and modern propagation techniques in *piper nigrum*. *J. Med. Plant Res*., 4(1): 7-12.

Ahmad, N., H. Fazal and B. H. Abbasi (2011). *In vitro*Larvicidal potential and Antioxidative enzymes activities in *Ginkgo biloba, Stevia rebaudiana* and *Partheniumhysterophorous*.*Asian Pacific Journal of Tropical Medicine*, 13 (4): 169-175.

Ahmad, N., H. Fazal, B. H. Abbasi and S. Farooq (2010). An efficient free radical scavenging activity in *Ginkgo biloba, Stevia rebaudiana* and *Partheniumhysterophorous* leaves through DPPH (2,2-diphenyl-1-picrylhydrazyl). *Inter. J. Phytomed*., 2: 231-239.

Ahmad, N., H. Fazal, B. H. Abbasi, I. U. Rahman, S. Anwar, M. A. Khan, A. Basir, H. Inayat, R. Zamir, S. A. Khalil and K. Y. Khan (2011). DPPH scavenging antioxidant potential in regenerated tissues of *Stevia rebaudiana, Citrus sinensis* and *Saccharumofficinarum*.*J. Med. Plant Res*.,5: 3293-3297.

Antia, B. S., E. J. Akpan, P. A. Okon and U. I. Umoren (2006).Proximate composition and phytochemical constituents of leaves of some *Acalypha* species.*Pak. J. Nutri*., 5: 166-168.

Association of Official Analytical chemists (AOAC). (1990). *Official Method Cd-8-53*.American Oil Chemistry Society, Champaign, IL.

Association of Official Analytical chemists (AOAC). (2003). *Official Methods of Analysis*.15thedn.Association of official analytical chemists. Arlington, Virginia.

Awen, B. Z., S. Ganapati and B. R. Chandu (2010). Influence of *Sapindus mukorossi* on the permeability of ethyl cellulose free film for transedermal use. *Res. J. Pharma. Biol. Chem. Sci*., 1: 35-38.

Aziz, S., S. Naher, M. D. Abukawsar and S. K. Roy (2012).Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of the Two Varieties of the Black Pepper (*Piper nigrum*Linn.). *Int. J. Pharm. Phyto-Pharm. Res*., 2(2): 67-70.

Bhat, S. R., K. P. S. Chandel and S. K. Malik (1995). Plant regeneration from various explants of cultivated *Piper* species. *Plant Cell Rep*., 14: 398-402.

Butt, M. S., I. Pasha, M. T. Sultan, M. A. Randhawa, F. Saeed and W. Ahmed (2013). Black pepper and health claims, a comprehensive treatise. Crit. Rev. *Food Sci. Nutr*., 53: 875-886.

Edeoga H. O., D. E. Okwu and B. O. Mbaebie (2003).Minerals and nutritive value of some Nigerian medicinal plants. *J. Med. Arom. Plant Sci.*, 25:1010-1015.

Enwere N. J. (1998). *Food of Plant Origin*. Afro Orbis Publishers, Nsukka, pp.22-30.

Gohari. R. N., O. Seyed, M. Fahimeh, M. Maryam, T. Shohreh, A. Hassan and S. Soodabeh (2012). Evaluation of the Cytotoxicity of *Saturejaspicigera* and its main compounds. *Sci. World J.*, Article ID 203861.

Hamrapurkar, P. D., K. Jadhav and S. Zine (2011).Quantitative Estimation of Piperine in *Piper nigrum* and *Piper longum* Using High Performance Thin Layer Chromatography. *J. App. Pharmaceut. Sci*., 1: 117-120.

Harris W. S. (1989). Fish oils and plasma lipid and lipoprotein metabolism in humans.*J. Lipid Res*. 30: 785–807.

Hossain, M. D., B. K. Paul, S. K. Roy, G. C. Saha, F. Begumand D. Huq (2014).Studies on Fatty Acids Composition and Some Valuable Nutrients of *Piper nigrum*Linn. (GolMorich). *Dhaka Univ. J. Sci*., 62(2): 65-68.

Hussain, A., S. Naz, H. Nazir and Z. K. Shinwari (2011). Tissue culture of Black pepper (*Piper nigrum* L..) in Pakistan. *Pak. J. Bot*., 43: 1069-1078.

Hussain, J., L. A. Khan, N. Rehman, Zainullah and F. Khan (2009). Proximate and nutrient investigations of selected medicinal plant species of Pakistan. *Pak. J. Nut*r., 8: 620-624.

Jacobs M. B., (2006). *The Chemical Analysis of Foods Products*.CBS Pub. Distr. Pvt Ltd. Ind., 3rd Ed.,365-383.

Kashif, M. and S. Ullah (2013).Chemical Composition and Minerals Analysis of *Hippophae rhamnoides, Azadirachta indica, Punica granatu* and *Ocimum sanctum* Leaves. *W. J. D. Food Sci*., 8(1): 67-73.

King C. G. (1970). Biological and medical aspects of fats.*J. Am. Oil Chem. Soc*., 47: p418.

Kochhar, K. P. (2008). Dietary spices in health and diseases : I. *Indian J. Physiol. Pharmacol.*, 52: 106-122.

Lee. S. (2005). *Encyclopedia of Chemical processing*. CRC Press.

Mir, M. A., M. Mustafa, B. A. Mir and A. Kumar (2014).Determination of Physicochemical Parameters of Fixed Oils of *Argemonemexicana*.L. *AJPR*., 4(2): 1539-1543.

Mowla G., N. M Sheick. and A. S. M. Kamal (1990). Hand Book on Edible Oils and Fats with Special Reference to Bangladesh.*Bang. J. Sci. Ind. Res*., 1stEd., 9-172.

Nair, R. R. and S. D. Gupta (2003). Somatic embryogenesis and plant regeneration in black pepper (*Piper nigrumL*.). direct somatic embryogenesis from tissue of germinating seeds and ontogeny of somatic embryos. *J. Hortic. Sci. Biotchnol*., 78: 416-421.

Nestel P. J. (1990). Effects of n-23 fatty acids on lipid metabolism.*Annu. Rev. Nutr*. 10:149-67.

Nollet L. M. L. (2004). *Hand book of Food analysis, Physical Characterization and Nutrient Analysis, Food Science and Technology*. CRC Press.2ndEd. 1: 221-274.

Nyam, K. L., C. P. Tan, O. M. Lai, K. Long and Y. B. Che-Man (2009). Physicochemical properties and bioactive compounds of selected seed oils.*Food Sci. Tech*., 42: 1396-1403.

Quan, L., S. F. Li, S. J. Tian, H. Xu, A. Q. Lin and L. Gu (2004). Determination of organochlorine pesticides residue in ginseng root by orthogonal array design Soxhlet extraction and gas chromatography. *Chromatographia*., 1: 89-93.

Rani, S. K., N. Saxena and Udaysree (2013). Antimicrobial activity of Black Pepper (*Piper nigrum* L.*).Glob. J. Pharmacol*., 7(1): 87-90.

Saleh-E-In M. M. and S. K. Roy (2007).Studies on Fatty Acid Composition and Proximate Analyses of *Anethum sowa* L. (Dill) Seed. *Bang. J. Sci. Ind. Res*., 42(4):455-464.

Santra, M., D. K. Santra, V. S. Rao, S. P. Taware and S. A. Tamhankar (2005). Inheritance of beta-carotene concentration in durum wheat (*Triticum turgidum* L.ssp.durum). *Eucalypta*, 144: 215-221.

Scott, I. M., H. R. Jensen, B. J. R. Philogene and J. T. Arnason (2008).A review of *Piper* spp. (Piperaceae), Phytochemistry, insecticidal activity and mode of action. *Phytochem. Rev*., 7: 65-75.

Singh, A. and S. Duggal (2009). Piperine-Review of Advances in Pharmacology. *Inter. J. Pharma. Sci. Nanotech*., 2: 615-620.

Srinivasan.K. (2007). Black pepper and its pungent principle-piperine, a review of diverse physiological effects. *Crit*. Rev. *Food Sci.Nutr*., 47: 735-748.

Sruthi, D., T. J. Zachariah, N. K. Leela and K. Jayarajan (2013). Correlation between chemical profiles of black pepper (*Piper nigrum*L.) var. Panniyur-1 collected from different locations. *J. Med. Plant Res*., 7(31): 2349-2357.

Szallasi, A. (2005). Piperine, researchers discover new flavor in an ancient spice. *Trends. Pharmacol. Sci.*, 26: 437-439.

Thomas, R. A. and S. Krishnakumari (2015). Proximate analysis and mineral composition of *Myristica fragrans* seeds. *J. Pharm. Phytocnem*., 3(6): 39-42.

Vadivel, V. and K. Janardhanan (2005).Nutritional and ant nutritional characteristics of seven South Indian wild legumes. *Plant Food Hum.Nutri*., 60: 69-75.

Zaveri, M. A., Khandhar, S. Patel and A. Patel (2010).Chemistry and pharmacology of *Piper longum* L. *Inter. J. Pharma. Sci. Rev. Res*., 5: 67-76.

(*Accepted for publication March 2024*)