Fatty Acid Composition, Functional Group Analysis and Antioxidant Activity of *Nymphia alba* and *Lupinus polyphyllus* Seed Extracts

Zubair Rehman Nengroo and Abdul Rauf*

Department of Chemistry, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, INDIA

Abstract: Seed extracts of *Nymphia alba* Linn. and *Lupinus polyphyllus* Lindl. were analyzed for fatty acid composition, functional group analysis and antioxidant activity. The petroleum ether extract of seeds were found dominant in unsaturated fatty acids with oleic acid (39.9%) and linoleic acid (29.6%) in *L. polyphyllus* and linoleic (37.5%) and oleic acid (10.9%) in *N. alba*. All the defatted seed extracts of *N. alba* and *L. polyphyllus* found to have powerful DPPH, ABTS, *H*₂*O*₃ and NBT antioxidant radical scavenging activity with reference to butylated hydroxy toluene (BHT). The defatted seed extracts were further analyzed with functional group analysis through FTIR found to contain numerous functional groups which may be responsible for their antioxidant activity.

Key words: fatty acid composition, functional group analysis, antioxidant activity, GC-MS, FTIR, oleic acid, linoleic acid, *Nymphia alba* Linn., *Lupinus polyphyllus* Lindl.

1 Introduction

Lipids are main constituents of man’s diet. They are found in all parts of plant, but mostly dominant in seeds. Some plants can provide oils with high concentration of unsaturated fatty acids, which can reduce cardiovascular diseases[1] and decreases the plasma lipids[2]. Lipids are used not only as fat emulsions for clinical nutrition but also as a carrier for various active substances. They are good source of polyphenols which protect the body from harmful free radicals, by activating endogenous immune system and modulating cellular processes[3]. Lipids are used in pharmaceutical and cosmetic fields as excipients, coadjuvants, transdermal carriers and skin emolliency agents[4]. Oleic acid can be blended with antioxidant to enhance the protective action, blends of oleic acid with tocopherol have better protective ability than individual tocopherol[5]. Oleic acid has neurotrophic effect on human brain[6]. During metabolic process and contact process with environment free radicals are produced in human body and if present at high concentration can cause oxidative stress thus damaging internal balance of human body which leads to serious diseases such as cancer, cardiovascular, neurodegenerative and premature aging[7, 8]. The use of plant extracts as natural antioxidants is the modern trend in research because plants usually contain wide range of free radical scavenging molecules such as phenolic components, nitrogen components, vitamins and many more[9–12], secondly damaging and toxic effect of synthetic antioxidants on human health[13–15]. Moreover, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are highly volatile and unstable at high temperature. Therefore, the aim of this work is to further continue our laboratory work[16–18], determination of fatty acid composition on seed oils of *Nymphia alba* Linn. and *Lupinus polyphyllus* Lindl. and further screening of various defatted seed extracts for functional group analysis and antioxidant activity. The characteristic properties of *N. alba* Linn. and *L. polyphyllus* Lindl. are shown in Table 1.

2 Materials and Methods

2.1 Chemicals and reagents

Petroleum ether(40–60°C), diethyl ether, absolute methanol(MeOH), ethanol(EtOH), n-hexane(purity 96%, HPLC grade) were purchased from Sigma-Aldrich chemicals (USA). Potassium hydroxide(KOH), sodium sulphate(Na₂SO₄) and sodium hydrogen carbonate(NaHCO₃) were purchased from TCI chemicals (India) and sulphuric acid(95–97%) was supplied by Merck chemicals and Reagents.

*Correspondence to: Abdul Rauf, Department of Chemistry, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, INDIA
E-mail: abdulrauchem@gmail.com

Accepted January 25, 2020 (received for review April 25, 2019)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

http://www.jstage.jst.go.jp/browse/jos/  http://mc.manuscriptcentral.com/jjocs
2.3 Characteristics of seed powder

2.3.1 Extraction of oil and extracts

The seeds of *N. alba* and *L. polyphyllus* were dried, grinded into powder and stored in opaque screwed tight jars until use. Powdered seeds of *N. alba* and *L. polyphyllus* were charged into soxhlet apparatus and extraction was carried out by using 160 mL of following solvents successively: (1) Petroleum ether (40-60°C), (2) Chloroform, (3) Ethyl acetate, (4) Acetone and (5) Methanol. The solvent in each extract was evaporated under vacuum by using rotary vacuum evaporator and dried residue was collected in opaque glass bottles for further studies. The oil characteristics were determined according to the standard AOCS procedures.

2.3.2 Preparation of fatty acid methyl esters (FAMEs)

The three step acid-catalyzed technique saponification, acidification and esterification was carried out according to method. One gram oil of *N. alba* and *L. polyphyllus* was saponified separately with 0.5N KOH in ethanol for about 3 h in water bath. The unsaponified part was removed with diethyl ether and saponified part was acidified with 6N HCl and extracted with diethyl ether to get mixed fatty acids (MFAs).

The MFAs were treated with excess absolute methanol and few drops of H₂SO₄ as catalyst and the reaction was refluxed for about 1 h. After completion of reaction monitored by thin layer chromatography (TLC), the resulting mixture was diluted with ice chilled water to get the cloud point. The resulting mixture was extracted with diethyl ether continuously. The combined extracts were washed with 5% aqueous sodium bicarbonate and dried over anhydrous sodium sulphate to yield FAMEs which were further purified with petroleum ether and diethyl ether (98.5/1.5, v/v) by column chromatography.

2.4 Chromatographic techniques

Thin layer chromatography was done on glass plates (10 cm × 5 cm) with a layer of silica gel (60-120 mesh, Merck, Mumbai, India, 0.5 mm thick). Petroleum ether-diethyl ether-acetic acid (80:20:1) was used as developing solvent. The spots of the synthesized FAMEs were observed in iodine vapor. Silica gel(Merck, Mumbai, India, 60-120 mesh) was used to carry out column chromatography.

2.5 ATR-FTIR analysis

Transmittance spectra of FAMEs and defatted seed extracts (chloroform, acetone, ethyl acetate and methanol) of *N. alba* and *L. polyphyllus* were obtained using a Perkin–Elmer Spectrum One FTIR spectrometer (UK) fitted with an Attenuated Total Reflectance (ATR) crystal of zinc selenide. The temperature of ATR crystal was maintained at 65°C, so that the sample which was put on it will cover whole crystal completely. A very small amount (50-100 µL) of the sample dissolve in carbon tetrachloride was used to cover surface area of the ATR crystal. The samples were measured in duplicate. The spectra were observed continuously over a wavelength range of 500–4000 cm⁻¹ with a data resolution of 4 cm⁻¹ and air was taken as a reference background material. After each scan, the ATR crystal was cleaned with tissue paper wet with ethanol and then dried.

2.6 GC/MS analysis

The fatty acid composition was determined by using Gas chromatography, Perkin Elmer (GC, Clarus 680) using an Elite-5MS capillary column (0.25 mm × 30 mm) with a flame ionization detector (FID) and mass spectrometer (MS). Helium was used as a carrier gas at a flow rate of 0.5 mL/min. The temperatures of the injector, column and detector are 180°C, 260°C and 280°C respectively. The oven temperature was programmed as follows: 180°C for 2 min, then raised to 200°C at 2°C/min, held at 200°C for a further 10 min.

### Table 1 Characteristics properties of worked plants.

| Name of plant       | Local name | Family       | Uses                                                                 |
|---------------------|------------|--------------|----------------------------------------------------------------------|
| *Nymphia alba* Linn.| Guli nilofar| Nymphaeaceae | *N. alba* have been used to treat various diseases. Its extracts of rhizomes and flowers have anti-diabetic and anti-inflammatory effects. Seed and rhizome extracts have immunomodulatory effects. Its seed extracts have hepatoprotective and radical scavenging effects. It has antibacterial, anti-diarrheal, anticancer and antiviral activities. |
| *Lupinus polyphyllus* Lindl. | Trum/Lopine | Leguminosae | Lupins are consumed by humans in Mediterranean and Andean regions. Lupin showing rotational and nematicidal activity. Lupin have great nutritional value. It is used as livestock feed for humans and cattle. Lupin plants are used to control soil pollution. |

(India).
Fatty Acid Composition, Functional Group Analysis and Antioxidant Activity of Seed Extracts

J. Oleo Sci. 69, (4) 317-326 (2020)

Absorbance sample
Absorbance control

Absorbance sample = 1 - \frac{Absorbance control}{Absorbance sample} \times 100

2.7.4 Nitroblue Tetrazolium assay (NBT assay)

Superoxide anion scavenging activity was determined as described by Vyas[30]. The reaction was performed in 50 mmol/L phosphate buffer (pH 7.8) containing concentrations (25-200 µg/mL) of the defatted seed extracts of *N. alba* and *L. polyphyllus* and 50 mmol/L nitroblue tetrazolium (NBT), 10 mmol/L D,L-methionine, and 0.025% (v/v) Triton X-100. The reaction was initiated by illuminating the reaction mixture, the absorbance of formazan was recorded at 560 nm, and the percentage scavenging activity was described as the inverse of the produced formazan. BHT was used as a positive control.

2.8 Statistical analysis

Experimental results were given as mean ± standard deviation of three separate experiments. Data were analyzed in Microsoft Excel 2010.

3 Result and Discussion

3.1 Physicochemical analysis of seed oils

The oil extracted through soxhlet extraction from seeds of *L. polyphyllus* and *N. alba* was found to be 8.4% and 4.2% respectively (Table 2). Saponification value (S.V) is the indicator of the average molecular weight and hence chain length[29]. Higher the saponification value lesser is the chain length of fatty acids in a triglyceride. The iodine value (I.V) gives the idea about degree of unsaturation which can be used to determine the oxidative stability of oils. As given in Table 2, high S.V (131.4) of *L. polyphyllus* as compared to *N. alba* (91.9) is mainly because of high content of low molecular chain acids in *L. polyphyllus* as compared to *N. alba*. The I.V of *L. polyphyllus* is 87.6 while as *N. alba* has 75.8. This difference may be mainly due to high content of unsaturated fatty acids 70.3% in *L. polyphyllus* as compared to *N. alba* 48.4% (Table 3).

3.2 Functional group analysis

FTIR spectra is one of the most important and widely used powerful tool for the determination of functional groups in various plant extracts. This technique works based on functional groups which gives information in the form of peak values. In this work ATR-FTIR analysis was used to observe different functional groups in defatted seed extracts (chloroform, acetone and methanol) of *N. alba* and *L. polyphyllus* by. As displayed in Fig. 1, the IR peaks of *N. alba* for defatted chloroform seed extract are

![Figure 1](https://example.com/fig1.png)

**Table 2**

| Saponification Value (S.V) | Iodine Value (I.V) |
|---------------------------|-------------------|
| *N. alba*                 | *L. polyphyllus*   |
| 91.9                      | 131.4              |

**Table 3**

| Functional Group | % of Total Fatty Acids |
|------------------|------------------------|
| Saturated        | 87.6                   |
| Unsaturated      | 12.4                   |
pointed while as acetone and chloroform extracts can be similarly taken and its IR peak values for functional groups are given in Table 4. Similarly, the FTIR peaks for L. polyphyllus for defatted chloroform and acetone are displayed in Fig. 2 and functional groups for particular extracts are given in Table 5. The wide IR in the range of 3200-3500 cm\(^{-1}\) shown by all extracts of two plants is mainly of –OH group which may be due to the presence of polyphenols, tocopherols, tocotrienols, vitamin E and C, flavonols or some other hydroxyl group containing antioxidants. The range of IR from 3000-3100 cm\(^{-1}\) is presence of unsaturated carbon, which could be due to presence of unsaturated antioxidants such as carotenoids (alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin and lycopene) or many more natural antioxidants. Similarly, the sharp peak in the range of 1740-1750 cm\(^{-1}\) could be mainly because of the presence of ester bearing natural antioxidants such as methionine esters etc. The peak at 1645-1655 cm\(^{-1}\) may be due to the presence of compounds such as cis beta carotene, z-stilbene etc. The ethereal functional group ranges in between 1250-1050 cm\(^{-1}\) could be because of presence of natural antioxidants such as vitamin C and E, flavonoids, anthocyanins etc. In general the FTIR analysis gives valuable information regarding general functional groups present in defatted seed extracts of worked plants, which could be very helpful for further work.

3.3 Fatty acid composition

The fatty acid composition is a good indicator of the
quality and stability of the oil. Therefore, determination of fatty acid composition is necessary. The seed oil of each plant was converted into fatty acid methyl ester (FAME) as required in GC-MS analysis. As given in Fig. 3, the peak at 1742 cm\(^{-1}\) is ester peak of \(N.\) \(alba\) and \(L.\) \(polyphyllus\) seed oils. The fatty acid composition was determined by Gas chromatography (GC) and various peaks were identified by retention time and mass spectra (MS). From Table 3 total unsaturated fatty acids were dominant acids in \(L.\) \(polyphyllus\) 70.3% and \(N.\) \(alba\) 48.4%. In \(N.\) \(alba\) linoleic acid 37.5% was most dominant fatty acid followed by oleic acid 10.9% which is comparably similar with other species of its family\(^{39}\). In case of \(L.\) \(polyphyllus\) oleic acid 39.9% was dominant fatty acid followed by linoleic acid 29.6% which is relatively similar with its other species\(^{40}\). The saturated fatty acids include palmitic acid (15.5%, 15.5%) and stearic acid (1.0%, 1.7%) for \(N.\) \(alba\) and \(L.\) \(polyphyllus\) respectively. Moreover \(N.\) \(alba\) contains significant amount of eicosanoic acid 7.3%. Other fatty acids were found in very little amount.

3.4 Antioxidant activity

A number of methods and modifications have been used to determine the antioxidant activity. DPPH radical scavenging, hydrogen peroxide, superoxide anion radical and hydroxyl radicals are widely used for this purpose\(^{41}\).

3.4.1 DPPH radical scavenging activity

DPPH radical scavenging activity as shown in Fig. 4. All the defatted seed extracts (chloroform, acetone, ethyl acetate and methanol) of \(N.\) \(alba\) and \(L.\) \(polyphyllus\) shows good DPPH radical scavenging activity, but most dominant inhibition of free radicals was shown at 100 µg/mL and 200 µg/mL. At 100 µg/mL with respect to butylated hydroxy toluene (BHT) having percent inhibition 81%, ethyl acetate extract of \(L.\) \(polyphyllus\) shows maximum inhibition of radicals 79% followed by 77% inhibition of acetone extract of \(L.\) \(polyphyllus\) and chloroform extract of \(N.\) \(alba\), followed by 76% inhibition each of acetone and methanol extracts of \(N.\) \(alba\). The other defatted seed extracts (chloroform, acetone and methanol) of \(L.\) \(polyphyllus\) showed less radical scavenging activity. At 200 µg/mL with respect to BHT having 94% inhibition the dominant DPPH radical scavenging was shown by defatted acetone extract 92% and ethyl acetate extract 85% inhibition of \(L.\) \(polyphyllus\) followed by methanol 84%, acetone

![Fig. 2 ATR-FTIR analysis of defatted chloroform, acetone and methanol extracts of \(L.\) \(polyphyllus\).](image)

### Table 4 FTIR analysis of defatted chloroform, acetone and methanol seed extracts of \(N.\) \(alba\).

| Chloroform Wavenumber cm\(^{-1}\) | Functional group | Acetone Wavenumber cm\(^{-1}\) | Functional group | Methanol Wavenumber cm\(^{-1}\) | Functional group |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 3408                            | Alcohol         | 3386            | Alcohol         | 3392            | Alcohol         |
| 3014                            | cis RHC=CHR     | 3014            | cis RHC=CHR     | 2924            | --CH\(_2\)--     |
| 2924                            | --CH\(_2\)--    | 2924            | --CH\(_2\)--    | 2856            | --CH\(_2\)--    |
| 2854                            | --CH\(_2\)--    | 2856            | --CH\(_2\)--    | 1718            | --C=O (acid)    |
| 2056                            | Non-assigned    | 1748            | --C=O (ester)   | 1618            | Non-assigned    |
| 1740                            | --C=O (ester)   | 1612            | Non-assigned    | 1464            | --C=O (CH\(_2\))|
| 1648                            | C=C cis-olefins | 1510            | --C=H (CH\(_2\))| 1234            | --C=O           |
| 1516                            | --C=H (CH\(_2\))| 1422            | --C=H (CH\(_2\))| 1054            | =CH\(_2\)       |
| 1460                            | --C=H (CH\(_2\))| 1366            | --C=H (CH\(_2\))| 874             |                 |
| 1378                            | --C=H (CH\(_2\))| 1282            | --C=H (CH\(_2\))|                 |                 |
| 1166                            | --C=O           | 1234            | --C=H (CH\(_2\))|                 |                 |
| 1076                            | --C=O           | 1146            | --C=O           |                 |                 |
| 974                             | --HC=CH\(_2\) (trans) | 1010          | --C=O           |                 |                 |
| 626                             | O--H            | 726             | --(CH\(_2\))\(_n\) |                 |                 |

Table was constituted according to References\(^{42–44}\).
The chloroform and methanol defatted seed extracts of *L. polyphyllus* shows 78% and 74% inhibition respectively.

### 3.4.2 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) radical scavenging activity

ABTS radical scavenging activity as shown in Fig. 5. Almost all defatted seed extracts of *N. alba* show dominant ABTS radical scavenging activity as compared to *L. polyphyllus*. The dominant effect was shown at 50 µg/mL, 100 µg/mL and 200 µg/mL concentration of defatted seed extracts of *N. alba* respectively; 5, 6, Eal, 7 are defatted (chloroform, acetone, ethyl acetate and methanol) seed extracts of *L. polyphyllus* respectively. BHT: butylated hydroxy toluene taken as positive control.

- **Table 5** FTIR analysis of defatted chloroform, acetone and methanol seed extracts of *L. polyphyllus*.

| Chloroform Wavenumber cm⁻¹ | Functional group | Acetone Wavenumber cm⁻¹ | Functional group | Methanol Wavenumber cm⁻¹ | Functional group |
|-----------------------------|------------------|-------------------------|------------------|--------------------------|------------------|
| 3504                         | Alcohol          | 3460                    | Alcohol          | 3402                     | Alcohol          |
| 2936                         | −CH₂−            | 2922                    | −CH₂−            | 2922                     | −CH₂−            |
| 2912                         | −CH₂−            | 2854                    | −CH₂−            | 2854                     | −CH₂−            |
| 1750                         | −C=O (ester)     | 1744                    | −C=O (ester)     | 1734                     | −C=O (ester)     |
| 1652                         | C=C cis-olefins  | 1699                    | Non-assigned     | 1618                     | Non-assigned     |
| 1460                         | −C=H (−CH₃)      | 1528                    | −C=H (−CH₃)      | 1448                     | −C=H (−CH₃)      |
| 1356                         | −C=H (−CH₃)      | 1356                    | −C=H (−CH₃)      | 1456                     | −C=H (−CH₃)      |
| 1242                         | −C=O            | 1242                    | −C=H (−CH₃)      | 1242                     | −C=H (−CH₃)      |
| 1106                         | −C=O            | 1174                    | −C=O            | 1051.00                  | −C=O            |
| 1164.98                      | −HC=CH− (cis)   | 1048.09                 | −C=O            | 1112                     | −C=O            |
| 1116.96                      | O=H             | 922                     | −HC=CH− (cis)   | 777.71                   | −C−H            |

Table was constituted according to References 42–44.

Fig. 3 ATR-FTIR analysis of FAMEs of *N. alba* and *L. polyphyllus*, the peak at 1742 cm⁻¹ is ester peak. CCl₄ is taken as solvent in analysis.

DPPH Radical Scavenging Activity

83%, chloroform 82% and ethyl acetate 80% inhibition. The chloroform and methanol defatted seed extracts of *L. polyphyllus* shows 78% and 74% inhibition respectively.

---

83%, chloroform 82% and ethyl acetate 80% inhibition. The chloroform and methanol defatted seed extracts of *L. polyphyllus* shows 78% and 74% inhibition respectively.

3.4.2 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) radical scavenging activity

ABTS radical scavenging activity as shown in Fig. 5. Almost all defatted seed extracts of *N. alba* show dominant ABTS radical scavenging activity as compared to *L. polyphyllus*. The dominant effect was shown at 50 µg/mL, 100 µg/mL and 200 µg/mL concentration of defatted seed ex-
tracts. At 200 µg/mL the most dominant effect was shown by chloroform, ethyl acetate, methanol and acetone extracts of N. alba with (86%, 85%, 82% and 77%) inhibition respectively with respect to BHT having 91% inhibition. L. polyphyllus shows 78% inhibition for ethyl acetate and 76% inhibition for chloroform, acetone and methanol extracts. At 100 µg/mL with BHT having 80% inhibition the defatted chloroform, methanol and acetone shows dominant (75%, 73% and 71%) inhibition respectively for N. alba. The ethyl acetate extract of L. polyphyllus shows 72% inhibition followed by chloroform 68% and acetone 67% inhibition of free radicals. At 50 µg/mL ethyl acetate extract of L. polyphyllus having 55% inhibition shows dominant effect followed by chloroform 54% and methanol 52% inhibition for N. alba with respect to BHT having 59% inhibition.

3.4.3 Hydrogen peroxide (H₂O₂) scavenging activity
H₂O₂ radical scavenging activity of defatted seed extracts shown in Fig. 6. The dominant scavenging was by defatted seed extracts at 100 µg/mL and 200 µg/mL. At 200 µg/mL with respect to BHT which showed 91% inhibition, the defatted methanol, chloroform and ethyl acetate extracts of L. polyphyllus shows most dominant (87, 82 and 80%) inhibition respectively followed by defatted methanol and ethyl acetate extracts of N. alba which shows 81% and 79% inhibition of radicals respectively. The defatted acetone seed extract of L. polyphyllus showed 75% inhibition while as chloroform and acetone extracts of N. alba shows 74% and 71% inhibition respectively. At 100 µg/mL L. polyphyllus shows dominant effect with defatted methanol, chloroform and ethyl acetate shows (75, 69 and 64%) inhibition of radicals respectively and methanol extract of N. alba shows 65% inhibition with respect to BHT having 84% inhibition. At 25 µg/mL and 50 µg/mL only methanol defatted seed extract of L. polyphyllus shows dominant effect with 30% and 62% inhibition of radical formation with reference to BHT having 32 and 68% inhibition respectively.

3.4.4 Nitroblue Tetrazolium assay (NBT assay)
NBT scavenging activity is shown in Fig. 7. The defatted chloroform, methanol seed extracts of N. alba and defatted seed extracts of ethyl acetate, methanol of L. polyphyllus shows dominant inhibition of radicals at all concentrations. At 50 µg/mL the chloroform and methanol defatted seed extracts of N. alba shows 56% and 46% inhibition of free radicals respectively, while ethyl acetate and methanol defatted extracts of L. polyphyllus shows 51% and 49% inhibition respectively with respect to standard BHT 65% inhibition. The other defatted extracts showed average inhibition. At 100 µg/mL with BHT having 83% inhibition the defatted chloroform, acetone and methanol extracts of N. alba showed 76%, 65% and 63% inhibition respectively while as defatted ethyl acetate and methanol extracts of L. polyphyllus showed 75% and 67% inhibition respectively. At 200 µg/mL with BHT having 92% inhibition the defatted chloroform, methanol, ethyl acetate and acetone extracts of N. alba shows 74% and 71% inhibition respectively. At 100 µg/mL L. polyphyllus shows dominant effect with defatted methanol, chloroform and ethyl acetate shows (75, 69 and 64%) inhibition of radicals respectively and methanol extract of N. alba shows 65% inhibition with respect to BHT having 84% inhibition. At 25 µg/mL and 50 µg/mL only methanol defatted seed extract of L. polyphyllus shows dominant effect with 30% and 62% inhibition of radical formation with reference to BHT having 32 and 68% inhibition respectively.

3.4.4 Nitroblue Tetrazolium assay (NBT assay)
NBT scavenging activity is shown in Fig. 7. The defatted chloroform, methanol seed extracts of N. alba and defatted seed extracts of ethyl acetate, methanol of L. polyphyllus shows dominant inhibition of radicals at all concentrations. At 50 µg/mL the chloroform and methanol defatted seed extracts of N. alba shows 56% and 46% inhibition of free radicals respectively, while ethyl acetate and methanol defatted extracts of L. polyphyllus shows 51% and 49% inhibition respectively with respect to standard BHT 65% inhibition. The other defatted extracts showed average inhibition. At 100 µg/mL with BHT having 83% inhibition the defatted chloroform, acetone and methanol extracts of N. alba showed 76%, 65% and 63% inhibition respectively while as defatted ethyl acetate and methanol extracts of L. polyphyllus showed 75% and 67% inhibition respectively. At 200 µg/mL with BHT having 92% inhibition the defatted chloroform, methanol, ethyl acetate and acetone extracts of N. alba shows 74% and 71% inhibition respectively. At 100 µg/mL L. polyphyllus shows dominant effect with defatted methanol, chloroform and ethyl acetate shows (75, 69 and 64%) inhibition of radicals respectively and methanol extract of N. alba shows 65% inhibition with respect to BHT having 84% inhibition. At 25 µg/mL and 50 µg/mL only methanol defatted seed extract of L. polyphyllus shows dominant effect with 30% and 62% inhibition of radical formation with reference to BHT having 32 and 68% inhibition respectively.

3.4.4 Nitroblue Tetrazolium assay (NBT assay)
NBT scavenging activity is shown in Fig. 7. The defatted chloroform, methanol seed extracts of N. alba and defatted seed extracts of ethyl acetate, methanol of L. polyphyllus shows dominant inhibition of radicals at all concentrations. At 50 µg/mL the chloroform and methanol defatted seed extracts of N. alba shows 56% and 46% inhibition of free radicals respectively, while ethyl acetate and methanol defatted extracts of L. polyphyllus shows 51% and 49% inhibition respectively with respect to standard BHT 65% inhibition. The other defatted extracts showed average inhibition. At 100 µg/mL with BHT having 83% inhibition the defatted chloroform, acetone and methanol extracts of N. alba showed 76%, 65% and 63% inhibition respectively while as defatted ethyl acetate and methanol extracts of L. polyphyllus showed 75% and 67% inhibition respectively. At 200 µg/mL with BHT having 92% inhibition the defatted chloroform, methanol, ethyl acetate and acetone extracts of N. alba shows 74% and 71% inhibition respectively. At 100 µg/mL L. polyphyllus shows dominant effect with defatted methanol, chloroform and ethyl acetate shows (75, 69 and 64%) inhibition of radicals respectively and methanol extract of N. alba shows 65% inhibition with respect to BHT having 84% inhibition. At 25 µg/mL and 50 µg/mL only methanol defatted seed extract of L. polyphyllus shows dominant effect with 30% and 62% inhibition of radical formation with reference to BHT having 32 and 68% inhibition respectively.

3.4.4 Nitroblue Tetrazolium assay (NBT assay)
NBT scavenging activity is shown in Fig. 7. The defatted chloroform, methanol seed extracts of N. alba and defatted seed extracts of ethyl acetate, methanol of L. polyphyllus shows dominant inhibition of radicals at all concentrations. At 50 µg/mL the chloroform and methanol defatted seed extracts of N. alba shows 56% and 46% inhibition of free radicals respectively, while ethyl acetate and methanol defatted extracts of L. polyphyllus shows 51% and 49% inhibition respectively with respect to standard BHT 65% inhibition. The other defatted extracts showed average inhibition. At 100 µg/mL with BHT having 83% inhibition the defatted chloroform, acetone and methanol extracts of N. alba showed 76%, 65% and 63% inhibition respectively while as defatted ethyl acetate and methanol extracts of L. polyphyllus showed 75% and 67% inhibition respectively. At 200 µg/mL with BHT having 92% inhibition the defatted chloroform, methanol, ethyl acetate and acetone extracts of N. alba shows 74% and 71% inhibition respectively. At 100 µg/mL L. polyphyllus shows dominant effect with defatted methanol, chloroform and ethyl acetate shows (75, 69 and 64%) inhibition of radicals respectively and methanol extract of N. alba shows 65% inhibition with respect to BHT having 84% inhibition. At 25 µg/mL and 50 µg/mL only methanol defatted seed extract of L. polyphyllus shows dominant effect with 30% and 62% inhibition of radical formation with reference to BHT having 32 and 68% inhibition respectively.
standard antioxidant drug butylated hydroxy toluene shows good antioxidant activities against \textit{L. polyphyllus} and \textit{N. alba} seed extracts, which can replace toxic synthetic drugs in industry especially in drug design and development with new processing and preservation techniques to fulfill the needs of modern society. Moreover the defatted seed extracts of \textit{N. alba} which contains high degree of unsaturated fatty acids which can make them useful in industry especially in foods.\textit{L. polyphyllus} shows 85\%, 81\%, 80\% and 79\% inhibition of free radicals.

4 Conclusion

There is recent trend in search of food and food items which contains less saturated fatty acids and natural drugs which can replace toxic synthetic drugs in industry and medicine because synthetic drugs are stable at particular conditions and over use of them leads to serious diseases. This work mainly emphasis on fatty acid composition of \textit{N. alba} and \textit{L. polyphyllus} which contains high degree of unsaturated fatty acids (health promoting oleic and linoleic acid)\textit{ which can make them useful in industry especially in foods}. Moreover the defatted seed extracts of \textit{N. alba} and \textit{L. polyphyllus} shows good antioxidant activities against standard antioxidant drug butylated hydroxy toluene (BHT), which can make them highly applicable in drug design and development with new processing and preservation techniques to fulfill the needs of modern society.

Acknowledgement

We are thankful to UGC for the award of Non-Net fellowship and Chairman, Department of Chemistry to provide necessary facilities to complete this article. We are also thankful to Prof. Sajad Ahmad Gangoo for helping in collection and identification of plant material.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1) Lopez-Miranda, J.; Badimon, L.; Bonanome, A.; Lainon, D.; Kris-Etherton, P.M.; Mata, P.; Perez-Jimenez, F. Monounsaturated fat and cardiovascular risk. \textit{Nutr. Rev.} \textbf{64}, S2-S12 (2006).
2) Muller, H.; Kirkhus, B.; Pedersen, J.I. Serum cholesterol predictive equations with special emphasis on trans and saturated fatty acids. An analysis from designed controlled studies. \textit{Lipids} \textbf{36}, 783-791 (2001).
3) Chen, C.; Yu, R.; Owuor, E.D.; Kong, A.N.T. Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. \textit{Arch. Pharm. Res.} \textbf{23}, 605-612 (2000).
4) Shahidi, F.; Senanayake, S.P.J.N. Nutraceutical and specialty lipids. in \textit{Nutraceutical and Specialty Lipids and Their Co-Products}. CRC press, Boca Raton, FL, pp. 2-25 (2006).
5) Lampi, A.M.; Kamal-Eldin, A. Effect of \(\alpha\)- and \(\gamma\)-tocopherols on thermal polymerization of purified high-oleic sunflower triacylglycerols. \textit{J. Am. Oil Chem. Soc.} \textbf{75}, 1699-1705 (1998).
6) Preedy, V.R.; Watson, R.R. eds. \textit{Olives and olive oil in health and disease prevention}. Academic Press (2010).
7) Gupta, R.K.; Patel, A.K.; Shah, N.; Chaudhary, A.; Jha, U.; Yadav, U.C.; Gupta, P.K.; Pakuwal, U. Oxidative stress and antioxidants in disease and cancer. \textit{Asian Pac. J. Cancer Prev.} \textbf{15}, 4405-4409 (2014).
8) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. \textit{Int. J. Biochem. Cell Biol.} \textbf{39}, 44-84 (2007).
9) Agati, G.; Azzarello, E.; Pollastrì, S.; Tattini, M. Flavonoids as antioxidants in plants: Location and functional significance. \textit{Plant Sci.} \textbf{196}, 67-76 (2012).
10) Cai, Y.; Sun, M.; Corke, H. Antioxidant activity of betalains from plants of the Amaranthaceae. \textit{J. Agric. Food Chem.} \textbf{51}, 2288-2294 (2003).
11) Chiang, C.J.; Kadouh, H.; Zhou, K. Phenolic compounds and antioxidant properties of gooseberry as
14. Azizkhani, M.; Zandi, P. Effects of some natural antioxidant mixtures on marigold stability. Pak. J. Agri. Sci. 47, 251-257 (2010).

15. Kowalski, R. GC analysis of changes in the fatty acid composition of sunflower and olive oils heated with quercetin, caffeic acid, protocatechuic acid, and butylated hydroxyanisole. Act. Chromatograph. 18, 15-23 (2007).

16. Ahmad, M.S.; Rauf, A.; Mustafa, J.; Sheikh, M.O. An 8-hydroxycotadec-11,14-dienoic acid from Mirabilis jalapa seed oil. Phytochemistry 23, 2247-2249 (1984).

17. Parveen, H.; Rauf, A. (Z)-12-Hydroxycotadec-9-enoic acid in Sesbania aculeata seed oil. Ind. Crops Prod. 27, 118-122 (2008).

18. Sharma, S.; Gangal, S.; Rauf, A. Lipase mediated hydrolysis of Mimusops elengi and Parkinsonia aculeata seed oils for the determination of positional distribution of fatty acids. Ind. Crops Prod. 30, 325-328 (2009).

19. Khan, N.; Sultana, S. Anticarcinogenic effect of Nymphaea alba against oxidative damage, hyperproliferative response and renal carcinogenesis in Wistar rats. Mol. Cell. Biochem. 271, 1-11 (2005).

20. Raja, M.M.M.; Sethiya, N.K.; Mishra, S.H. A comprehensive review on Nymphaea stellata: A traditionally used bitter. J. Adv. Pharm. Technol. Res. 1, 311-319 (2010).

21. Mukherjee, D.; Khatua, T.N.; Venkatesh, P.; Saha, B.P.; Mukherjee, P.K. Immunomodulatory potential of rizome and seed extracts of Nelumbo nucifera Gaertn. J. Ethnopharmacol. 128, 490-494 (2010).

22. handarkar, M.R.; Khan, A. Antihepatotoxic effect of Nymphaea stellata Willd., against carbon tetrachloride-induced hepatic damage in albino rats. J. Ethnopharmacol. 91, 61-64 (2004).

23. Sohn, D.H.; Kim, Y.C.; Oh, S.H.; Park, E.J.; Li, X.; Lee, B.H. Hepatoprotective and free radical scavenging effects of Nelumbo nucifera. Phytomedicine 10, 165-169 (2003).

24. Bose, A.; Sahoo, M.; Ray, S.D. In vivo evaluation of anti-diarrheal activity of the rhizome of Nymphaea alba (Nymphaeaceae). Orient. Pharm. Exp. Med. 12, 129-134 (2012).

25. Thippeswamy, B.S.; Mishra, B.; Veerapur, V.P.; Gupta, G. Anxiolytic activity of Nymphaea alba Linn. in mice as experimental models of anxiety. Ind. J. Pharmacol. 43, 50-55 (2011).

26. Petterson, D.S. Chapter 12: Composition and food uses of Lupins. in Lupins as crop plants: biology, production and utilization (Gladstones, J.S.; Atkins, C.A.; Hamblin, J. eds.), CAB International, Wallingford, UK, pp. 353-384 (1998).

27. Yildiz, S. Rotational and nematicidal effect of lupine (Lupinus albus L. Leguminosae). Afr. J. Biotechnol. 10, 13252-13255 (2011).

28. Engedaw, L.Y. Potential of lupins (Lupinus spp. L.) for human use and livestock feed in Ethiopia. Koster, 1st ed. Auflage, Koster, Berlin, Germany, p. 198 (2012).

29. Kitessa, S.M. The nutritional value of Russell lupin (Lupinus polyphyllus & Lupinus arboresus) for sheep. Masters theases, Lincoln University, New Zealand (1992).

30. Vazquez, S.; Agha, R.; Granado, A.; Sarro, M.J.; Esteban, E.; Penalosa, J.M.; Carpena, R.O. Use of white lupin plant for phytoestabilization of Cd and As polluted soil. Water Air Soil Pollut. 177, 349-365 (2006).

31. Link, W.E. Off. Tent. Method. Amer. Oil Chem. Soc. 3rd ed. AOCS Champ. 11, USA. Methods Da 15-48 and Da 16-48 (1973).

32. Morshed, M.; Ferdous, K.; Khan, M R.; Mazumder, M.S.I.; Islam, M.A.; Uddin, M.T. Rubber seed oil as a potential source for biodiesel production in Bangladesh. Fuel 90, 2981-2986 (2011).

33. Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agri. Food Chem. 40, 945-948 (1992).

34. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice- Evans, C. Antioxidant activity applying an improved ABTS radical decolorization assay. Free Rad. Bio. Med. 26, 1231-1237 (1999).

35. Ruch, R.J.; Cheng, S.J.; Klumig, J.E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen. 10, 1003-1008 (1989).

36. Vyas, D.; Kumar, S. Purification and partial characterization of a low temperature responsive Mn-SOD from tea (Camellia sinensis (L.) O. Kuntze). Biochem. Biophys. Res. Commun. 329, 831-838 (2005).

37. Nehdi, I.A.; Shibi, H.; Tan, C.P.; Zarrouk, H.; Khalil, M.I.; Al-Resayes, S.I. The nutritional value of Russell lupin (Lupinus polyphyllus & Lupinus arboresus) for sheep. Masters theases, Lincoln University, New Zealand (1992).

38. Hunter, J.E. Studies on effects of dietary fatty acids as related to their position on triglycerides. Lipids 36, 655-668 (2001).

39. Aliyu, M.; Kano, M.A.; Abdullahi, N.; Kankara, I.A.;
Ibrahim, S.I.; Muhammad, Y.Y. Extraction, characterization and fatty acids profiles of *Nymphaea Lotus* and *Nymphaea Pubescens* seed oils. *Biosci. Biotech. Res. Asia* **14**, 1299-1307 (2017).

40) Khalid, I.I.; Elhardallou, S.B. Physico-chemical properties and fatty acids composition of bitter and sweet lupine seed. *Orien. J. Chem.* **35**, 1148-1153 (2019).

41) Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **40**, 945-948 (1992).

42) Guillen, M.D.; Cabo, N. Characterization of edible oils and lard by Fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. *J. Am. Oil Chem. Soc.* **74**, 1281-1286 (1997).

43) Silverstein, R.M.; Bassler, G.C.; Kiemle, D.J. *Spectroscopic identification of organic compounds*. 7th ed. John Wiley & Sons, Hoboken, USA (Chapter 2) (2005).

44) Vlachos, N.; Skopelitis, Y.; Psaroudaki, M.; Konstantinidou, V.; Chatzilazarou, A.; Tegou, E. Applications of Fourier transform-infrared spectroscopy to edible oils. *Anal. Chim. Acta* **573**, 459-465 (2006).