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OPTIMIZED METHANOLIC EXTRACT OF MEDICINALLY IMPORTANT FAGONIA INDICA IMPROVED THE OXIDATIVE STABILITY OF SUNFLOWER OIL AT ACCELERATED STORAGE

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Abstract

The oxidative deterioration of vegetable oils is a leading cause to impart various diseases in consumers. The synthetic antioxidants used to stabilize the fats and oils are proved to be toxic for human consumption. Alternatively, medicinal plants provide a significant deal of vital metabolites to encounter food oriented oxidative damages to human body and edible oils. In current study different organic solvents of variable polarity were used to optimize the extraction from Fagonia indica (F. indica) and to evaluate its impact on 1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), total phenolic contents (TPC) and total flavonoid contents (TFC). The methanolic extract yielded comparative high extract amount of 24.18±0.36%, TPC 184.17±3.28 mg/g GAE, TFC 102.04±1.02 mg/g RE, DPPH scavenging 94.84±0.28% and FRAP value of 303.55±5.05 TE mM/mL. Being the most potent, methanolic extract was blended with refined, bleached and deodorized sunflower oil in various concentrations. The stabilized sunflower oil samples were stored at 65 ± 2 °C in electric oven for six days and their free fatty acid value, peroxide value, iodine value, ρ-anisidine value, conjugated dienes and trienes were determined after regular interval of 24 hours. The FTIR analysis provided noticeable bands of intensities related to oxidation of sunflower oil to evaluate the extent of deterioration. The values of rancidity parameters were compared with blank and butylated hydroxyanisole blended sunflower oil

samples. The methanolic extract of F. indica at 600 mg/kg of oil substantially delayed the oxidative deterioration of sunflower oil during six days heating protocol. F. indica may be presented as a vital source of novel antioxidant system to enhance the shelf life, oxidative stability and bio-functionality of vegetable oils making it safe for human consumption and health improvement.

Keywords: Fagonia indica, antioxidant, oxidative stability, sunflower oil, accelerated storage, FTIR analysis.

INTRODUCTION

The crude edible oils are not acceptable to consumers due to taste and sensory properties. Crude or raw vegetable oils are refined to remove some problem creating substances including phosphor lipids, metals, free fatty acids and color causing agents. Due to high degree of unsaturation, vegetable oils undergo phenomenon of lipid oxidation which imparts off flavor and toxicity, rendering them unsuitable for human consumption (Mishra et al., 2021). The most of the vegetable oils contain polyunsaturated fatty acids having unsaturation sites susceptible to oxidation (Maurelli et al., 2009). Both the long storage time and high temperature processing favors oxidation process to impart rancidity and nutrition loss due to production of free radicals in oils and oil containing foods (Soriguer et al., 2003).

Free radicals can be encountered effectively using compounds known as antioxidants. Antioxidants possess the ability to delay the processes of primary and secondary lipid oxidation by capturing the free radicals. Various synthetic antioxidants are being used to inhibit lipid oxidation in oils and fats during storage and processing. Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butyl hydroquinone (TBHQ) are under frequent use to reduce the pace of oxidation reaction in edible oils. Use of BHA, BHT and TBHQ has been condemned for human consumption due to associated toxicity (Amakura et al., 2002; Orhanet al.,2003; Shahidi, 2005). Japan, Canada and Europe have banned the TBHQ as antioxidant in food systems. Similarly BHA has been eliminated from generally recognized as safe (GRAS) compound list due to toxicity issues (Frag et al., 1998). Therefore researchers are inclined towards searching antioxidant of natural origin due to their safety and acceptability among consumers. Plants serve as rich source of natural antioxidants and nutraceuticals due to presence of biologically active ingredients like polyphenols and flavonoids (William et al., 2019; Raza et al., 2021). Phenolic compounds are believed as best antioxidants due to significantly higher redox potential and protect the lipid molecules or break the chain of oxidation reaction (Laguerre et al., 2015). Many plants have been searched for potent antioxidant role and still many plants need to be explored in this regards. The plant sources are recommended as excellent source of pharmacologically active ingredients to combat oxidative stress and microbial growth (Arooj et al., 2023). The major identified components in plant extracts are usually tocopherols, ascorbic acid, carotenoids and other phenolic compounds. These compounds are responsible for the medicinal as well as antioxidant activities of plants (Ali et al., 2016; Vichiet al.,2001). Vegetable oils stabilized with various plant extracts, have been investigated for their oxidative stability at ambient and elevated temperatures to assess the antioxidant activities of plant ingredients (Ali et al., 2016; Anwar et al., 2010; Raza et al., 2009). Frying and cooking involved high temperatures leading to rapid increases in rate of oxidative alteration by producing free radicals. Deep frying is highly used method for food processing and protective role of antioxidants at elevated temperatures in vegetable oils is therefore necessarily checked to monitor the oxidative stability of oil with and without plant extracts (Raza et al., 2014; Ali et al., 2016). So plants can serve as innovative free radical scavenging tool to control the oxidation reaction in vegetable oil model.

Fagonia indica (*F. indica*) is a well-known medicinal herb native to India and Pakistan being frequently used to treat many ailments (Ali et al., 2008; Azam et al., 2019). The antioxidant potential of aerial parts of *F. indica* is well established and its major phytochemical constituents are flavonoids, saponins, tannins and glycosides (Latif et al., 2019; Javed et al., 2021). The strong

antioxidant activities of *F. indica* urged to optimize the extraction process for better extract yield and to evaluate its impact on free radical scavenging activity, total phenolic contents and total flavonoid contents. The most potent fraction was utilized to stabilize the refined, bleached and deodorized (RBD) sunflower oil for oxidative stability assessment at ambient and accelerated storage.

MATERIALS AND METHODS

Chemicals and reagents

Aluminum chloride ($AlCl_3$), Sodium hydroxide (NaOH), 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Sodium nitrite ($NaNO_2$), Folin-Ciocalteu reagent. The chemicals and reagents used in study were of high quality and purity (Merck, Dramstadt, Germany).

Collection and processing of plant material

Fresh plant material was collected from area of Dina tehsil of District Jhelum, Pakistan and immediately quenched in liquid nitrogen. The plant material was identified from Department of Botany, GC University Lahore, Pakistan. The aerial parts of F. indica were powdered in mortar and pestle and fine powdery material was sieved and stored at very low temperature for further use.

Extract preparation

The fine powdery plant material was immersed in methanol, ethanol, ethyl acetate, chloroform and n-hexane for 24 hours, shaken for 2 hours on mechanical shaker followed by30 min ultrasonication. The debris was removed through filtration and extra solvent was evaporated using vacuum generated evaporation equipped with temperature control. The semi-solid extracts were weighed for extract yield calculation and stored in freezer till further use.

Total phenolic contents (TPC)

Established method was used by adopting minute changes. Briefly 20 µl of each extract was mixed in 90 µl of Folin-Ciocalteu reagent. Resulting mixture was added 91 µl of 10% Na_2CO_3. The absorbance was measured at 726 nm using LABOMED, INC spectro UV-VIS double beam, UVD-3000. Gallic acid was used as standard reference and final values were reported in equivalence to gallic acid (GAE mg/g) for each extract (Zenginet al., 2010).

Total flavonoid contents (TFC)

An already reported method was adopted for determination of TFC in extracts of F. indica with slight modifications (Kim et al., 2003). Extracts were dissolved in methanol followed by adding freshly prepared 0.5 M $NaNO_2$ (0.10mL). Afterwards, $AlCl_3.6H_2O$ (0.3 M) and MeOH (30%) were added to make up the final volume of 200 µL. After 5 min stay at ambient conditions the reaction mixture was made alkaline using NaOH standard solution. The spectrophotometric measurement was taken as absorbance on selected absorbance of 510 nm. Results were calculated as milligrams of rutin equivalent in each gram of understudy dried plant extracts (mg RE/g DE) as rutin was taken as standard flavonoid for standard curve preparation.

DPPH activity

Various F. indica extracts were subjected to DPPH radical scavenging by adopting a previously reported method with minute changes (Mensor et al., 2001). The plant extracts in measured quantity were added to freshly prepared methanolic DPPH reagent. A continuous shaking for thorough mixing was carried out for 10 min for further incubation at room temperature in absence of light (25 min). The final absorbance at 517 nm for DPPH color bleaching was taken. The percent scavenging was calculated by following formula.

$$\text{Scavenging \%} = \frac{(\text{Absorbace of Control} - \text{Absorbance of Sample})}{\text{Absorbance of control}} \times 100$$

BHA was used as standard synthetic antioxidant. The analysis was performed in triplicate.

Ferric reducing antioxidant power assay (FRAP)

A well-established method for FRAP activity was selected to determine antioxidant activity of extracts (Benzie and Strain, 1996). Acetate buffer having pH of 3.6 was prepared by mixing sodium acetate and acetic acid. The acidic solution of TPTZ (2,4,6-tripyridyl-s-triazine) was prepared using 40 mM of standard HCl followed by adding further 20 mM solution of analytical grade $FeCl_3.6H_2O$. Fresh solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ and $FeCl_3.6H_2O$. The resultant solution was heated at 375°C for further utilization. Plant extracts and Trolox were mixed together and 10 µL of each sample solution was mixed with FRAP reagent (3 mL). The final samples were allowed to stay in dark for almost 30 min time period for complex formation. The absorbance of reaction was taken at 593 nm on spectrophotometer. Findings were calculated based upon Troloxequivalent (TE mM/mL).

Oxidative stability of stabilized SFO by Schaal oven test

The SFO samples were stabilized with 200, 400 and 600 mg of plant extract per kg of oil. The SFO having BHA (200 mg/kg) were also run under the same experimental protocol. SFO with no additive was treated as blank. Stabilized SFO samples were stored at 65 ± 2 °C in oven for six days to determine free fatty acids (FFA %), peroxide value (PV meq O_2. Kg^- oil), iodine value (IV), p-anisidine value (PAVmg/Kg) and ultra-violet absorption analysis after every 24 hours to measure the extent of oxidation. The FFA % (Ca 5a-40), PV (Cd 8-53) and p-anisidine value (Cd 18-90) were determined by recommended methods of American Oil Chemist's Society (AOCS, 1998). Ultraviolet spectrophotometric indices were determined as specific extinction coefficients K_{232} at 232 nm and K_{268} at 268 nm (Ch 5-91) by official methods of AOCS (AOCS 1998).

FTIR analysis

Fourier transform infrared (FTIR) spectra were recorded using KBr discs with sample size of 20 µl using a Spectrum II spectrometer by Perkin Elmer (Waltham, MA, USA) with 40 scans with interval of 4 cm^{-1} in IR region 4000-625 cm^{-1}. The attenuated total reflectance (ATR) mode was used (Liang et al., 2013).

Statistical analysis

The standard deviation was applied for triplicate values of results and one way analysis of variance (ANOVA) was applied to compute statistical significance using Minitab 17.0 software.

RESULTS AND DISCUSSION

Effect of solvent on polyphenols and antioxidant activity

The results of extract yield, TPC, TFC, DPPH assay and FRAP assay are given in Table 1. The solvent system significantly differentiated extract yield, TPC, TFC and antioxidant activities. The methanol yielded highest extract yield, TPC and TFC as compared to other solvents used for extraction and the values were significantly higher as indicated by statistical analysis ($\rho<0.05$).

The extraction optimization is highly required to explore plant metabolites and high pharmacological throughput. The high TPC and TFC values for methanol extract might be due to high compatibility of phytochemicals with solvent. The role of solvent polarity was decided as major contributor towards high extract yields and polyphenols. The high TPC and TFC were reported as major constituents of plants responsible for antioxidant and medicinal potential (Do QD et al., 2014).

The antioxidant potential of F. indica extracts were assessed by DPPH radical scavenging assay and FRAP assay. The DPPH radical scavenging assay is based upon the stabilization phenomenon by the polyphenolic compounds usually present in plant extracts. The values of DPPH and FRAP assay indicated that all extracts reflected considerable antioxidant activities but methanol extract of F. indica exhibited maximum antioxidant activities and found significantly higher among all extracts ($\rho<0.05$). The high antioxidant activities of methanolic extract were based upon the high phenolic contents. The role of solvent used for extraction was of immense significance to obtain high antioxidant activities. The methanol was reported as best optimal choice for extraction of polyphenols from various plant parts to enhance the antioxidant potential for better output (Farooq et al., 2020, Raza et al., 2020). The pivotal role of plant extracts being rich in polyphenolic contents to prevent food deterioration from oxidative damages and microbes was reported as an established fact (Papuc et al., 2017). Diets rich in polyphenols were reported to improve the defense system of human body against health disorders due to their antioxidant role and posed the plants as natural and safe source for food fortification (Loizzo and Tundis, 2019).

The statistical analysis revealed that methanol showed significant extract yield, TPC and TFC.

Table 1: Effect of solvent on extract yield, TPC, TFC and antioxidant activities of F. indica

Solvent	Extract yield %	TPC mg/g GAE	TFC mg/g RE	DPPH scavenging %	FRAP (TE mM/mL)
Ethanol	22.52±0.44 ^{b}	166.22±3.96 ^{b}	77.10±2.05 ^{b}	92.11±0.22 ^{b}	277.72±4.94 ^{b}
Methanol	24.18±0.36^{a}	184.17±3.28 ^{a}	102.04±1.02 ^{a}	94.84±0.28 ^{a}	303.55±5.05 ^{a}
Ethyl acetate	17.91±0.31 ^{c}	102.50±2.25 ^{d}	68.92±2.24 ^{c}	87.60±0.30 ^{c}	208.81±3.33 ^{c}
Chloroform	16.88±0.22 ^{cd}	111.38±2.18 ^{c}	55.57±1.13 ^{d}	82.95±0.15 ^{d}	195.47±4.16 ^{d}
n-hexane	13.33±0.11 ^{e}	87.20±1.05 ^{e}	42.25±1.75 ^{e}	77.06±0.09 ^{e}	178.02±3.08 ^{e}

Oxidative stability of SFO

The results of rancidity parameters including FFA, PV, IV, and p-anisidine value of blank and stabilized SFO samples are given in Fig. 1.

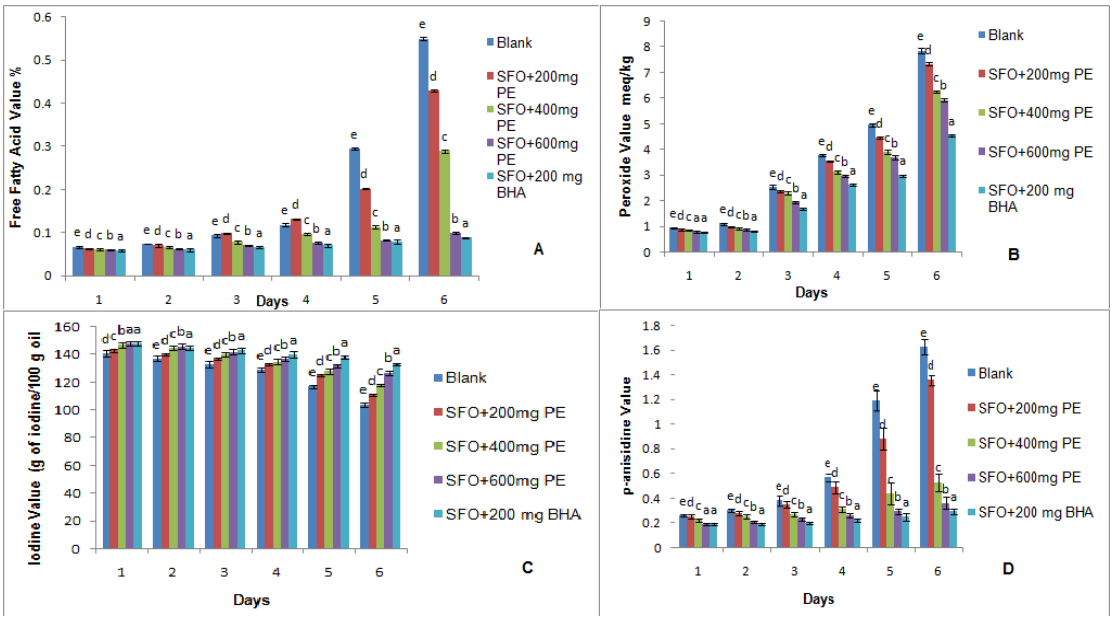

Fig 1. Free fatty acid value, peroxide value, iodine value, ρ-anisidine value of SFO samples. Values do not sharing a letter are statistically significant ($\rho<0.05$).

The FFA are produced as a result of oxidation and hydrolysis of vegetable oils and the process is augmented at higher temperature. The FFA values of blank SFO were increased from 0.067±0.002% to 0.55±0.004% at the end of six days heating protocol. The plant extract at dose of 600 mg/kg oil restricted the rapid increase in FFA formation at the end of six days period (0.10±0.02%) and quite comparable with the SFO sample having 200 mg/kg BHA for which the final value was 0.088±0.002%. However statistical analysis revealed a significant difference between plant extract stabilized SFO and BHA stabilized SFO ($\rho>0.05$).

Peroxides are the first product of oxidation process and reported to cause serious health ailments like cardiovascular disorders, allergies and obesity (Pizzino et al., 2017). There was no significant difference observed in PV of SFO having 600 mg/kg plant extract and BHA containing SFO after first day of heating. But with the passage of heating period, the PV were increased to considerable extent for blank SFO with final reading of 7.86±0.11 meq/kg. The plant extract at 600 mg/kg oil and BHA at 200 mg/kg oil substantially restricted the increase of PV with final values of 5.93±0.08 meq/kg and 4.55±0.05 meq/kg, respectively. The peroxide values were increased with the passage of heating period as an indicator of oxidative damage to SFO substrate. The IV is the indication of degree of unsaturation of vegetable oils. The results indicated that a decrease of 37 was observed for blank SFO after six days heating period. Where a decrease of 21 in IV was observed for SFO sample having 600 mg of plant extract and a decrease of 15 was noticed for SFO blended with BHA. The ρ-anisidine value reflected the aldehydic secondary oxidation products of fats and fats containing foods (Anwar et al., 2007). The results indicated that ρ-anisidine value increased with heating and this increase was more rapid during the last two days heating period reflecting the magnitude of secondary oxidation process. However, plant extract at 600 mg/kg oil restricted the increase in ρ-anisidine value of SFO to reasonable extent and was much better than blank SFO, SFO±200 mg and SFO±400 mg extract containing samples. The value of ρ-anisidine for SFO±600 mg PE was closer to SFO±200 mg BHA but statistical analysis indicated that the SFO±600 mg PE was more susceptible to secondary oxidation ($\rho>0.05$).

The CD and CT were measured in terms of molar extinction coefficient at 232nm and 268 nm, respectively. The values of CD and CT were reported as good indicators to assess the level of oxidation in vegetable oils (El-Hadary and Taha 2020). The results of CD (Table 2) and CT (Table 3) indicated a typical trend in CD and CT values of blank SFO where, the plant extracts showed a promising restriction in CD and CT increase over consecutive heating period. A comparable finding was noticed for CD and CT of SFO±600 mg PE and SFO±200 mg BHA. Other concentrations of extracts also slowed down the oxidation process but not so remarkably as was in the case of SFO±600 mg PE.

The findings of oxidative stability parameters confirmed the protective role of plant extract of F. indica against oxidative damage to SFO at accelerated storage by imparting antioxidant role. The significant antioxidant potential of F. indica extract was most probably attributed to the high amounts of polyphenolic compounds which were also indicated in TPC and TFC results. The current investigation suggested that the antioxidant potential of F. indica to delay the rancidity of SFO was in dose dependent manner. Some recent reports also highlighted the plant extracts as valuable source of natural antioxidants to stabilize the vegetable oils rather than using synthetic antioxidants in safer and healthy diet perspectives (Meng et al., 2021; Blasi and Cossignani 2020; Raza et al., 2014). The F. indica exhibited good antioxidant activity and contained high amounts of polyphenols which were responsible for the improved oxidative stability of cooking grade sunflower oil. The lipids and lipids containing food systems may be improvised by incorporating F. indica extracts to solve the issues of shelf life and oxidative stability associated with fats.

Table 2: Conjugated dienes (CD) values of treated SFO samples and blank under accelerated storage.

No of Days	Blank	SFO+200 mg PE	SFO+400 mg PE	SFO+600 mg PE	SFO+200 mg BHA
1	0.22±0.01 ^{f}	0.22±0.01 ^{ef}	0.20±0.01 ^{e}	0.20±0.01 ^{e}	0.20±0.01 ^{e}
2	0.25±0.01 ^{e}	0.24±0.01 ^{e}	0.20±0.01 ^{e}	0.20±0.01 ^{e}	0.20±0.01 ^{e}
3	0.35±0.02 ^{d}	0.31±0.02 ^{d}	0.26±0.03 ^{d}	0.22±0.01 ^{d}	0.20±0.01 ^{e}
4	0.51±0.03 ^{c}	0.45±0.03 ^{c}	0.37±0.03 ^{c}	0.26±0.02 ^{c}	0.24±0.01 ^{c}
5	0.69±0.03 ^{b}	0.60±0.05 ^{b}	0.49±0.03 ^{b}	0.33±0.03 ^{b}	0.28±0.02 ^{b}
6	0.91±0.04 ^{a}	0.79±0.03 ^{a}	0.64±0.03 ^{a}	0.40±0.02 ^{a}	0.36±0.03 ^{a}

Table 3: Conjugated trienes (CT) values of treated SFO samples and blank under accelerated storage.

No of Days	Blank	SFO+200 mg PE	SFO+400 mg PE	SFO+600 mg PE	SFO+200 mg BHA
1	0.10±0.01 ^{e}	0.10±0.01 ^{e}	0.08±0.01 ^{f}	0.06±0.01 ^{f}	0.06±0.01 ^{e}
2	0.10±0.01 ^{e}	0.10±0.01 ^{e}	0.09±0.01 ^{e}	0.06±0.01 ^{e}	0.07±0.01 ^{d}
3	0.15±0.01 ^{d}	0.11±0.01 ^{d}	0.11±0.02 ^{d}	0.08±0.01 ^{d}	0.07±0.01 ^{d}
4	0.18±0.01 ^{c}	0.15±0.01 ^{c}	0.13±0.01 ^{c}	0.11±0.02 ^{c}	0.10±0.01 ^{c}
5	0.29±0.03 ^{b}	0.21±0.03 ^{b}	0.18±0.03 ^{b}	0.15±0.03 ^{b}	0.13±0.02 ^{b}
6	0.37±0.01 ^{a}	0.30±0.03 ^{a}	0.26±0.03 ^{a}	0.20±0.02 ^{a}	0.16±0.03 ^{a}

FTIR analysis results

The comparison of FTIR spectra of SFO samples is given as Fig. 2. The spectra interpretation provided useful evidences on structure oriented chemical changes of SFO during heating. Stretching vibration of C-O ester group was noticed at 1173 cm^{-1}. The peaks at 1466 cm^{-1} were observed for all SFO samples due to –C-H(CH_2 and CH_3) bending vibrations. A discriminative trend was observed at peak 1746 cm^{-1}. This peak was due to formation of saturated aldehydic functional group and became more pronounced in blank SFO sample as compared to other SFO samples. This phenomenon was most probably reflected the high degree of oxidation in blank SFO having no additive or antioxidant. Peak at 2854 cm^{-1} was due to oxidation product response of symmetric stretching of –CH(CH_2). A sharp peak at 3379 cm^{-1} was observed only in case of blank SFO which was due to hydroxyl group of hydroperoxides formed from oxidative deterioration of polyunsaturated fatty acids (Roby et al., 2015). The FTIR spectra provided very useful information upon the initiation of secondary oxidation process on prolonged heating. Moreover SFO samples preserved with plant extract especially at concentration of 600 mg exhibited similar FTIR spectrum as was in case of SFO4. This fact also established a potent antioxidant activity of F. indica which was quite comparable with SFO4 having BHA (Liang et al., 2013; Rexhepi et al., 2019).

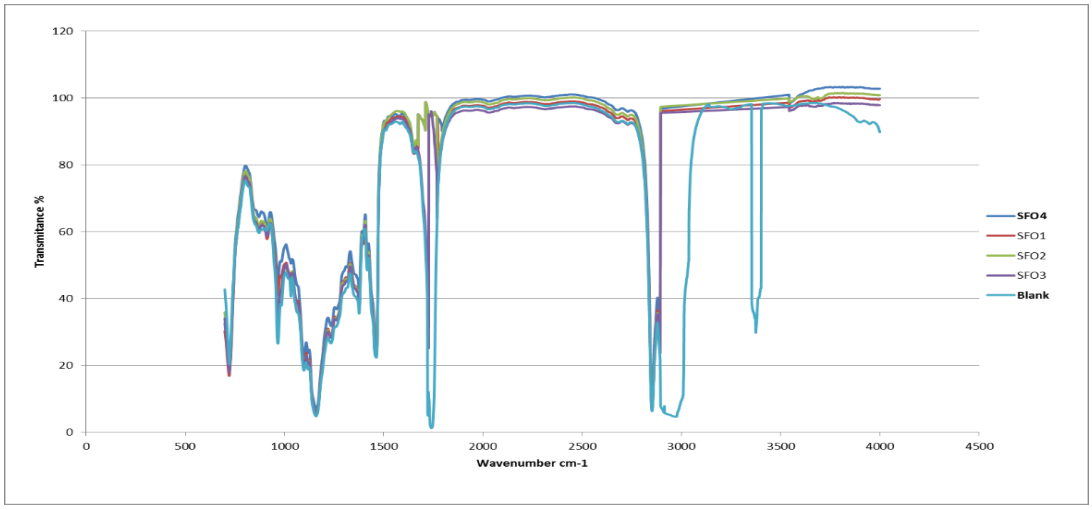

Fig 2. Comparison of FTIR spectra of SFO samples (SFO1 =. 200mg PE, SFO2 = 400 mg PE, SFO3 = 600 mg PE, SFO4 = 200 mg BHA)

CONCLUSION

The current study revealed that methanol was proved as best solvent extract bioactive metabolites from F. indica. Methanolic extract displayed significantly higher extract yield, antioxidant activities, total phenolic and flavonoid content values. The methanolic extract considerably delayed the oxidation of sunflower oil at elevated temperature. The FTIR analysis also revealed the mechanistic changes reflecting the oxidative deterioration of SFO under different treatment conditions. The findings proved F. indica as a rich source of natural antioxidants to enhance the oxidative stability of vegetable oils susceptible to deterioration due to high degree of unsaturation. Optimized fractionation may be adopted to narrow down the targeted compounds to achieve medicinally important pool of antioxidant metabolites for consumer health improvement and safety.

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