Study of Variations in the Extraction Yield, Phenolic Contents and Antioxidant Activities of the Bark of *F. religiosa* as a Function of Extraction Procedure

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Abstract: In present study, the effect of different solvents and multiple techniques on the extraction yield, phenolic contents and antioxidant activities of bark of *F. Religiosa,* was investigated. Four solvents (absolute ethanol, absolute methanol, 80% aqueous ethanol and 80% aqueous methanol) and three techniques (orbital shaker, sonication and magnetic stirrer) were applied for this purpose. The extract obtained by the application of 80% methanol, exhibited significantly ($p<0.05$) higher antioxidant activities. The statistical order of the solvents according to their efficiency was 80% methanol > 80% ethanol > absolute methanol >absolute ethanol. As for as techniques are concerned, the extracts obtained by the application of sonication demonstrated significantly ($p<0.05$) higher antioxidant activities as compared to to the extracts obtained as a result of using magnetic stirrer and orbital shaker.

Keywords: Antioxidant activities, DPPH radical, *Ficus religiosa*, total phenolic contents.

1. INTRODUCTION

The composition of phenolic compounds in extracts obtained from plants is affected by many factors including pre-treatment of sample, polarity of the solvent applied for extraction [1]; ratio of the extraction solvent to the plant material, extraction technique, chemical nature of phenolic compounds present in the plant and interfering compounds [2]. The solubility variation of phenolic compounds in different solvents is due to their structural diversification. Because of this limitation, extraction of phenolic compounds from plant material is largely dependent on the type of solvent. Polarity of the solvent affects the solubility of phenolic compounds [2-4]. Several organic solvents like ethanol, ethyl acetate, methanol, acetone and their aqueous combinations have been used for the extraction of phenolic compounds [1,5-14]. Selection of an appropriate and effective technique is also important to recover maximum amount of extractable phenolic compounds from plant matrices. Typical techniques used for this purpose include refluxing, soxhlet, orbital shaker, magnetic stirrer and maceration [14-22]. Other modern techniques reported for the extraction of phenolic compounds include super critical fluid extraction (SCFE), pressurized fluid extraction, sonication and microwave assisted extractions [4,23,24]. In recent years applications of ultrasound-assisted extraction for the extraction of phenolic components from different parts (fruit, leaves, stalk) have been studied [25-27].

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F. religiosa bark is widely used in the local medicine system and generally used as anti-inflammatory, antioxidant, anticonvulsant, and antidiabetic [28-30]. Presence of different chemical compounds including phenolic acids, flavonoids, saponins and tannins has been reported in the bark of *F. religiosa*. However, no literature has been found which explains the effect of different solvents and techniques on the extraction yield, phenolic contents and antioxidant activities of the bark of *F. religiosa*. The present research was designed to study the effects of different solvents and techniques on the extraction yield, phenolic contents and antioxidant activities of bark of *F. religiosa*; as it is important to apply appropriate extraction procedure for isolation of maximum amount of phytochemicals from plant matrix.

2. MATERIALS AND METHODS

Chemicals and reagents used during this research work were of analytical grade. All the chemicals (analytical grade) i.e. acetic acid, anhydrous sodium carbonate, sodium hydroxide, sodium nitrite, ammonium thiocyanate, ferrous chloride, potassium dihydrogen phosphate, aluminum chloride, dipotassium hydrogen phosphate, potassium iodide, and sodium thiosulphate used in this study were purchased from local distributer of Merck (Darmstadt, Germany) in Pakistan, unless stated otherwise.

2.1. Sample Collection

Barks of the *F. religiosa* were collected in the third week of April, from the botanical garden of University of

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Agriculture, Faisalabad. The specimen were further identified and authenticated by taxonomist Dr.Mansoor Ahmed, associate professor Department of Botany, University of Agriculture, Faisalabad, Pakistan. The collected samples were cleaned, dried in oven at 45 °C until constant mass achieved. The dried samples were ground by using a grinder (TSK-949, Westpoint, France) and then stored in a refrigerator at 4 °C for investigation.

2.2. Extraction Procedure

To evaluate the effect of extraction technique on the extraction yield and other antioxidant properties of the plant extracts, three different techniques (Orbital shaker, Magnetic stirring and Ultrasound extraction) were applied using four solvent systems i.e. 100% ethanol, 100% methanol, 80% ethanol (ethanol: water, 80:20 v/v), and 80% methanol (methanol: water, 80:20 v/v) separately in each technique.

2.3. Determination of Total Phenolic Contents (TPC)

Total phenolic contents of the samples were assessed by using the method described previously by Singleton *et al*., (1965) [31] and later followed by many researchers [4, 32, 33].

2.4. Determination of Total Flavonoid Contents (TFC)

The TFC of the extracts from the samples was determined by using spectrophotometric method as described previously by Sultana *et al*. (2008) [32] with slight modifications in concentrations according to the requirements.

2.5. DPPH. Radical Scavenging Assay

Free radical scavenging activity of the samples was determined by using 1, 1'–diphenyl–2-picrylhydrazyl (DPPH) method as reported earlier [33].

IC_{50} (Extract concentration providing 50% inhibition) for each sample was calculated by plotting graph of %age inhibitions against different concentrations of same sample.

2.6. Determination of Antioxidant Activity in Linoleic Acid System

The antioxidant activity of the sample extracts was also assessed by measuring the percent inhibition of linoleic acid oxidation [34].

2.7. Determination of Reducing Power

To determine the reducing power of the samples under investigation the method described byYen *et al*., (2000) [35], was used with slight modification.

2.8. Statistical Analysis

Three sample of *F. religiosa* bark was assayed. Each sample was analysed individually in triplicate for their extraction yield, phenolic contents and antioxidant potential. The data has been reported as mean (n = 1 x 3 x 3) ± standard deviation and analysed by analysis of variance (ANOVA) using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennysalvania, USA) at 5% significance level.

3. RESULTS AND DISCUSSION

Yields of extractable components from the bark samples varied significantly ($p \leq 0.05$) under the influence of different solvent systems applied for extraction. Data in the Figure **1** revealed that significantly ($p \leq 0.05$) higher yields were obtained when 80% ethanol was used as solvent. Significantly higher yields ($p \leq 0.05$) were obtained with sonication assisted extraction as compared to other two techniques (orbital shaking and magnetic stirring) applied for extraction. The combination of sonication assisted extraction technique and 80% ethanol as extraction solvent provided significantly higher ($p \leq 0.05$) yields in comparison to all other combinations of extraction technique and solvents used in the present study. Yields of extractable components from the bark of *F. religiosa* improved significantly($p \leq 0.05$) from 9.48±0.45 g/100g DW with least effective extraction system (absolute methanol and orbital shaker) to 13.91±0.66 g/100g DW with most effective extraction system (80% ethanol and sonication). Sultana *et al*., (2009)). [1] conducted a study to evaluate the effect of extraction procedure on the extraction yield from barks of *Azadirachta indica*, *Acacia nilotica, Eugenia jambolana*, *Terminalia arjuna* by applying the same solvents which were used in this study. In agreement with the results of present study they found that 80% ethanol extracted maximum amount of extractable components from the bark samples. In fact, the polarity of solvent has greater effect on the solubility of different components present in the plant. Many researchers concluded that higher yields of extract could be achieved by using more polar solvent [36, 37]. We obtained the highest yield with sonication assisted extraction which is subject to maintain the solvent composition. Many researchers

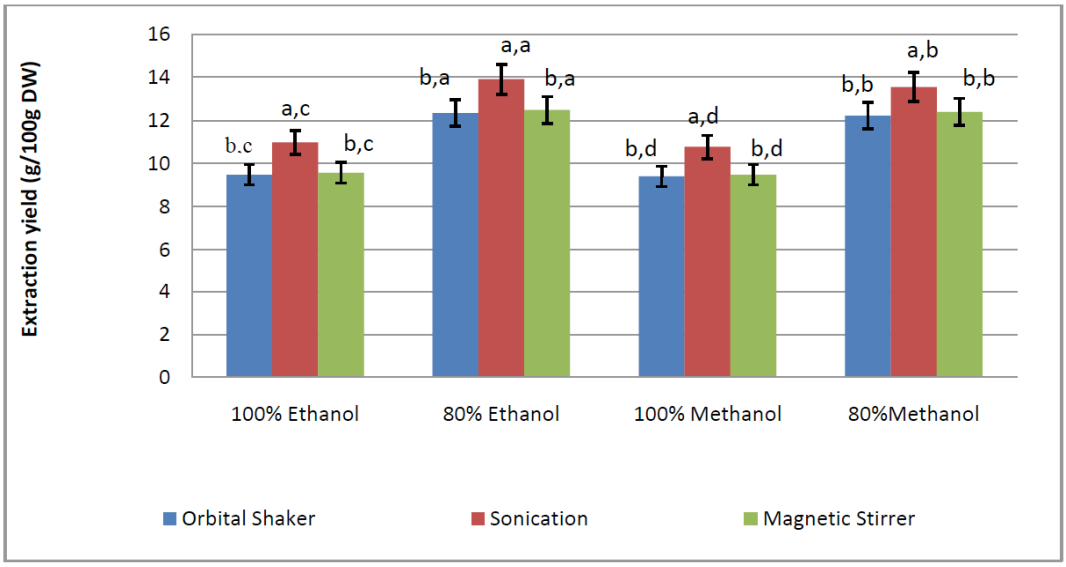

Figure 1: Effect of different solvents and techniques on the extraction yield of *F. religiosa* bark.

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD). **First letter above each bar represent different significance levels ($p \leq 0.05$) among different extraction technique and seconed letter above each bar represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

applied this technique on different plant material like soybeans [38] wheat bran [39] and coconut shell powder [40] and found that sonication was an effective tool for the extraction of bioactive components from plant materials.

The results presented in Table **1** revealed that the combination of 80% methanol with sonication was the most efficient method and the extracts obtained through this combination constituted significantly ($p<0.05$) higher amounts of total phenolic contents (TPC). Among the solvents, 80% methanol was the most effective solvents and the extracts obtained by the application of 80% methanol contained significantly ($p<0.05$) higher amounts of total phenolic in comparison with the extracts obtained by the application of other solvents used in the present study. The order of extraction efficiency of solvents on the basis of TPC was noted to be 80%methanol > 80% ethanol > 100%methanol>100% ethanol. The extracts obtained by using sonication gave significantly ($p<0.05$) higher amount of TPC. The total phenolic contents in the extract of the bark of *F. religiosa* increased from 3.90±0.16 g/100 g DW to 6.75±0.28 g/100 g DW while applying the least efficient combination of solvent and technique (absolute ethanol and orbital shaker) to the most efficient combination (80% methanol and sonication) applied. Anandjiwala *et al.*, (2008)[41] reported TPC by using 100% methanol as solvent in the stem bark of *F. religiosa* equal to 7.89±0.01% (w/w). The reported value for the stem bark of *F. religiosa* was higher than our result for the stem bark of

Table 1: Effect of Extraction Procedure on the Total Phenolic Contents (GAE g/100g of Dried Sample) and Total Flavonoids (CE g/100g of Dried Sample)

	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
TPC	Orbital Shaker	$3.90\pm0.16_b^d$	$5.44\pm0.23_b^b$	$4.14\pm0.17_b^c$	$6.56\pm0.28_b^a$
	Sonication	$4.03\pm0.17_a^d$	$5.59\pm0.23_a^b$	$4.25\pm0.18_a^c$	$6.75\pm0.28_a^a$
	Magnetic Stirrer	$3.94\pm0.17_b^d$	$5.45\pm0.23_b^b$	$4.14\pm0.17_b^c$	$6.57\pm0.28_b^a$
TFC	Orbital Shaker	$1.00\pm0.03_b^d$	$1.25\pm0.06_b^b$	$1.04\pm0.05_b^c$	$1.39\pm0.07_b^a$
	Sonication	$1.03\pm0.05_a^d$	$1.29\pm0.06_a^b$	$1.07\pm0.05_a^c$	$1.43\pm0.07_a^a$
	Magnetic Stirrer	$1.01\pm0.05_b^d$	$1.26\pm0.06_b^b$	$1.04\pm0.05_b^c$	$1.43\pm0.07_b^a$

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

F. religiosa 4.25±0.19 GAE g/ 100g DW. Many researchers calculated TPC from different parts of plants and most of them reported significantly higher values of TPC obtained by using polar solvents [42, 43].

Comparison of the results showed that sonication extraction technique was the most efficient than other two extraction techniques used for the recovery of total flavonoids (TF) while magnetic stirring was more efficient than orbital shaker. In most of the cases, amounts of total flavonoid obtained by the application of magnetic stirrer and orbital shaker were not significantly ($p<0.05$) different from each other. If we compare the efficiency of the solvent for the extraction of TF then order of efficiency in most of the samples was as follows: 80% methanol > 80% ethanol > absolute methanol >absolute ethanol. The analysis of bark samples revealed that extraction technique and solvent has appreciable effects on the yield of TFC. As a function of extraction solvent and techniques employed, the amount of TFC for bark samples varied from 1±0.03 to 1.43±0.07 CE g/100g DW for *F. religiosa,.* These trends observed in the present study regarding the efficiency of extraction solvent for recovery of total flavonoids from bark of *Ficus* species, are in close agreement to those recorded by [4,44] for extraction of flavonoids from different botanical materials using the same solvents for extraction.

DPPH radical scavenging capacity of extracts produced by different solvents and extraction techniques from the bark samples of *Ficus religiosa* in terms of IC_{50} values was given in Table **2**. According to the data, IC_{50} values improved significantly when different combinations of solvent with technique were employed for extraction. Radical scavenging activity (IC_{50} value) improved from 70.5±2.96 to 48.21±2.02 for *F. religiosa* by the application of different combinations of solvent with technique. Significantly ($p<0.05$) different radical scavenging activities obtained by the application of different solvents and statistical ranking of solvents on the basis of their DPPH radical scavenging activity was 80% methanol> 80% ethanol>100% methanol> 100% ethanol. IC_{50} values obtained by the application of orbital shaker and magnetic stirrer from the barks of *F. religiosa* were not significantly ($p<0.05$) different from each other.

Significantly ($p<0.05$) different reducing power was exhibited by the bark sample of the tested species under the influence of different solvents and techniques applied for extraction in the present study. The statistical ranking of solvents on the basis of the reducing power of extracts obtained by their application was 80% methanol > 80% ethanol > 100% methanol > 100% ethanol and the ranking for techniques was sonication > magnetic stirrer > orbital shaker. Reducing power of the bark samples varied over a wide range when we move from the least efficient combination (ethanol with orbital shaker) to the most efficient combination (methanol with sonication). Reducing power ranged from 0.61±0.03 to 1.02±0.04 for the bark of *F. religiosa*. All the lower values were significantly ($p<0.05$) different from their respective higher values.

Results calculated for the inhibition of linoleic acid peroxidation of the bark sample of *F. religiosa* are presented in Table **2** and these results confirm that the bark sample studied follow the general trend as

Table 2: Effect of Extraction Procedure on Antioxidant Activities

	Technique	100% Ethanol	80% Ethanol	100% Methanol	80% Methanol
DPPH radical scavenging activity (IC_{50}µg/mL)	Orbital Shaker	$70.5±2.96_a^a$	$57.30±2.41_a^c$	$68.95±2.89_a^b$	$49.65±2.09_a^d$
	Sonication	$69.55±2.92_b^a$	$56.05±2.35_b^c$	$68.05±2.86_b^b$	$48.21±2.02_b^d$
	Magnetic Strirer	$70.20±2.95_a^a$	$57.15±2.40_a^c$	$68.91±2.89_a^b$	$49.60±2.08_a^d$
Reducing power (absorbance at 700 nm)	Orbital Shaker	$0.61±0.03_c^d$	$0.84±0.04_c^b$	$0.63±0.03_c^c$	$0.99±0.04_c^a$
	Sonication	$0.63±0.03_a^d$	$0.86±0.04_a^b$	$0.67±0.03_a^c$	$1.02±0.04_a^a$
	Magnetic Strirer	$0.62±0.03_b^d$	$0.84±0.04_b^b$	$0.65±0.03_b^c$	$1.00±0.04_b^a$
%age inhibition of peroxidation in linoleic acid	Orbital Shaker	$53.61±2.52_c^d$	$54.92±2.58_c^b$	$53.96±2.54_c^c$	$58.59±2.75_c^a$
	Sonication	$55.15±2.59_a^d$	$56.18±2.64_a^b$	$55.52±2.61_a^c$	$60.28±2.83_a^a$
	Magnetic Strirer	$54.58±2.57_b^d$	$55.59±2.61_b^b$	$54.94±2.58_b^c$	$59.65±2.80_b^a$

*All the values in table are average of three values obtained after the analysis of sample in triplicate (n=1x3) and represented as (mean ± SD).
**Subscripts in a column represent different significance levels ($p \leq 0.05$) among different extraction technique and superscripts along the rows represent different significance levels ($p \leq 0.05$) among different solvents applied by LSD (least significant difference) test.

explained previously. Values of %age inhibition ranged from 53.61±2.52 to 60.28±2.83 for the extracts obtained from barks of *F. religiosa*. Zahid *et al.,* (2012) [44] investigated different antioxidant activities including DPPH radical scavenging activity, reducing power and inhibition of peroxidation in linoleic acid of the bark, fruit and leaves of *Pongamiapinnata* under the influence of different solvents including the solvents used in the present study and got the similar results regarding the efficiency of solvents. Shabir *et al.,* (2011) [45] studied effect of solvents on antioxidant activities (DPPH radical scavenging activity, reducing power and inhibition of peroxidation in linoleic acid) and reported that efficiency of different solvents was in the order of 80% methanol > 80% ethanol > absolute methanol >absolute ethanol and this finding was in agreement to the results concluded in the present study about the efficiency of different solvents.

CONCLUSIONS

The results of the present research clearly indicate that the extract obtained by the application of 80% methanol with sonication as extraction technique constitutes significantly ($p<0.05$) higher amounts of TPC and TFC and exhibit greater antioxidant activities in comparison to the extracts obtained by the application of other combinations of solvents with other techniques. This may be happened due to higher polarity of aqueous methanole solution and greater force applied by ultrasonic waves.

REFERENCES

[1] Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009; 14: 2167-80. http://dx.doi.org/10.3390/molecules14062167

[2] Shahidi F, Nackz M. Nutritional and pharmacological effect of food phenolics In: Shahidi F, Nackz M. editors. Foods and neutraceutical. CRC press NewYork 2004; pp. 331-402.

[3] Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry 2000; 55: 481-504. http://dx.doi.org/10.1016/S0031-9422(00)00235-1

[4] Zubair M, Anwar F, Shahid SA. Effect of extraction solvents on phenolics and antioxidant activity of selected varieties of Pakistani rice (*Oryza sativa* L.). Int J Agric Biol 2012; 14: 935-40.

[5] Adesegun SA, Fajana A, Orabueze CI, Coker HAB. Evaluation of antioxidant properties of *Phaulopsisfascisepala* C.B.C.l. (Acanthaceae). CAM 2007; 6: 227-31.

[6] Arabshahi-Delouee S, Urooj A. Antioxidant properties of various solvent extractsof mulberry (Morusindica L.) leaves. Food Chem 2007; 102: 1233-40. http://dx.doi.org/10.1016/j.foodchem.2006.07.013

[7] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocher P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem 2006; 97: 654-60. http://dx.doi.org/10.1016/j.foodchem.2005.04.028

[8] Dukic NM, Simin N, Cvejic J, Jovin E, Orcic D, Bozin B. Phenolic compounds in field Horsetail (*Equisetum arvense*L.) as natural antioxidant. Molecules 2008; 13: 1455-65. http://dx.doi.org/10.3390/molecules13071455

[9] Manzoor M, Anwar F, Nazamid S, Ashraf M. Variations of Antioxidant Characteristics and Mineral Contents in Pulp and Peel of Different Apple (*Malus domestica*Borkh.) Cultivars from Pakistan. Molecules 2012; 17: 390-407. http://dx.doi.org/10.3390/molecules17010390

[10] Miliauskas G, Venskutonis PR, Beek TAV. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85: 231-37. http://dx.doi.org/10.1016/j.foodchem.2003.05.007

[11] Potchoo Y, Guissou IP, Lompo M, Sakie E, Yaro B. Antioxidant activity of methanol and ethyl acetate extract of leaves of *Annona seneglensispers*from Togo versus the one originates from Burkina Faso. Int J Pharmacol 2008; 4: 67-77. http://dx.doi.org/10.3923/ijp.2008.120.124

[12] Rajeshwar Y, Kumar GPS, Gupta M, Mazumder UK. Studies on *in vitro* antioxidant activities of methanol extract of *mucunapruriens* (Fabaceae) seeds.Europ Bulletin of Drug Res 2005; 13: 31-7.

[13] Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J of Agricand Food Chem 2003; 51: 2866-87. http://dx.doi.org/10.1021/jf026182t

[14] Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R. Antioxidant activity, total phenolic and total flavonoid of extracts and fractions of red fruit (*Pandanusconoideus* Lam.). Int Food Res 2010; 17: 97-106.

[15] Sharififar F, Nudeh GD, Mitrajaldini M. Major flavanoids with antioxidant activity from *Teucriumpolium* L. Food Chem 2009; 112: 885-8. http://dx.doi.org/10.1016/j.foodchem.2008.06.064

[16] Bhalodi M, Shukla S, Saliya AK. *In vitro* antioxidant activity of the flower of Ipomoea aquatica Forsk. Pharmacogonsy Magazine 2008; 4: 226-30.

[17] Chahardehi AM, Ibrahim D, Sulaiman SF. Antioxidant Activity and Total Phenolic Content of Some Medicinal Plants in Urticaceae Family. J Applied Biol Sci 2009; 3: 25-9.

[18] Jayakumar T, Thomas PA, Geraldine P. *In vitro* antioxidant activities of anethanolic extract of the oyster mushroom *Pleurotusostreatus*. Innov Food Sci Emerg Technol 2009; 10: 228-34. http://dx.doi.org/10.1016/j.ifset.2008.07.002

[19] Kelen M, Tepe B. Screening of antioxidant properties and total phenolic compounds of various extracts of three different seed of grape varieties (*Vitisvinifera*L.) from Turkish flora. Pak J Bio Sci 2007; 10: 403-8. http://dx.doi.org/10.3923/pjbs.2007.403.8

[20] Motlhanka DMT. Free radical scavenging activity of selected medicinal plants of Eastern Botswana. Pak J Bio Sci 2008; 11: 805-8. http://dx.doi.org/10.3923/pjbs.2008.805.808

[21] Tian F, Li B, Ji B, Yang J, Zhang G, Che Y, Luo Y. Antioxidant and antimicrobial activities of consecutive extracts from *Gallachinensis*: the polarity has an effect on the bioactivities. Food Chem 2009; 113: 173-9. http://dx.doi.org/10.1016/j.foodchem.2008.07.062

[22] Vaidya SK, Viuswanalha GL, Ramesh C, Nandakumar K, Srinath R. Antimutagenic (Anticlastogenic) and antioxidant activities of bark extract of *Terminalia arjuna*. J Genetic Toxicol 2008; 1: 1-7.

[23] Rafael CD, Leite NM, Barbosa NR. Quantification of phenolic constituents and antioxidant activity of *Pterodonemarginatus* Vogel Seeds. Int J Mol Sci 2008; 9: 606-14. http://dx.doi.org/10.3390/ijms9040606

[24] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15: 7313-52. http://dx.doi.org/10.3390/molecules15107313

[25] Montiel-Herrera M, Camacho-HernándezI L, Ríos-Morgan A, Delgado-Vargas F. Partial physicochemical and nutritional characterization of the fruit of Vitexmollis (Verbenaceae). J of Food Comp Ana 2004; 17: 205-15. http://dx.doi.org/10.1016/j.jfca.2003.09.001

[26] Paniwnyk L, Cai H, Albu S, Mason TJ, Cole R. The enhancement and scale up of the extraction of anti-oxidants from Rosmarinus officinalis using ultrasound. Ultrason Sonochem 2009; 16: 287-92. http://dx.doi.org/10.1016/j.ultsonch.2008.06.007

[27] Yang Y, Gu D, Wu H, Aisa H, Zhang T, Ito Y. Application of preparative high-speed countercurrent chromatography for separation of elatine from *Delphinium shawurense*. J Liq Chrom Rel Technol 2008; 31: 3012-19.

[28] Singh D, Singh B, Goel RK. Traditional uses, Phytochemistry and Pharmacology of Ficus religiosa. J Ethanopharmacol 2011; 134: 565-583. http://dx.doi.org/10.1016/j.jep.2011.01.046

[29] Pandit R, Phadke A, Jagtap A. Antidiabetic effect of Ficus religiosa extract in streptozotocin-induced diabetic rats. J Ethnopharmacol 2010; 128: 462-466. http://dx.doi.org/10.1016/j.jep.2010.01.025

[30] Verma N, Chaudhary S, Garg VK, Tyagi S. Antiinflammatory and analgesic activity of methanolic extract of stem bark of Ficus religiosa. International Journal of Pharma Professional's Research 2010; 1: 145-147.

[31] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybadic-phosphotungstic acid reagents. Am J Enol Viticul 1965; 16: 144-55.

[32] Sultana B, Anwar F. Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem 2008; 108: 879-84. http://dx.doi.org/10.1016/j.foodchem.2007.11.053

[33] Anwar F, Ali M, Hussain AI, Shahid M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare Mill*.) seeds from Pakistan. Flav Frag J 2009; 24: 170-6.

[34] Iqbal S, Bhanger MI, Anwer F. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. LWT- Food Sci Tec 2007; 40: 361-7.

[35] Yen GC, Duh PD, Chuang DY, Murata N, Yamada M, Nishida I, Okuyama H, Sekiya J, Hajime W. Antioxidant activity of anthraquinones and anthrone. Food Chem 2000; 70: 437-41. http://dx.doi.org/10.1016/S0308-8146(00)00108-4

[36] Anwar F, Bhanger MI, Yasmeen S. Antioxidant activity of some natural extracts in corn oil.In: Murata N, Yamada M, Okuyama H editors. Advanced Research on Plant Lipid Springer Netherlands 2003; pp. 24-7. http://dx.doi.org/10.1007/978-94-017-0159-4_5

[37] Siddhuraju P, Becker K. Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringaoleifera Lam.) leaves. J of Agri and Food Chem 2003; 51: 2144-55. http://dx.doi.org/10.1021/jf020444+

[38] Rostagno MA, Palma M, Barroso CG. Solid-phase extraction of soy isoflavons. J of Chrom 2005; 1076: 110-7. http://dx.doi.org/10.1016/j.chroma.2005.04.045

[39] Wang H, Zhao M, Yang Jiang B, Rao G. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chem 2008; 107: 1399-406. http://dx.doi.org/10.1016/j.foodchem.2007.09.068

[40] Rodrigues S, Pinto GAS. Ultra sound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. J Food Eng 2007; 80: 869-72. http://dx.doi.org/10.1016/j.jfoodeng.2006.08.009

[41] Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of the free radical scavenging activity of an ayurvedic formula Panchvalkal. Indian J Pharm Sci 2008; 70: 31-5. http://dx.doi.org/10.4103/0250-474X.40328

[42] Bucić-Kojić A, Planinić M, Tomas S, Jakobek L, Šeruga M. Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. Int J Food Sci Technol 2009; 44: 2394-401. http://dx.doi.org/10.1111/j.1365-2621.2008.01876.x

[43] Spigno G, Tramelli L, Faveri DM. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J Food Eng 2007; 81: 200-8. http://dx.doi.org/10.1016/j.jfoodeng.2006.10.021

[44] Zahid IS, Anwar F, Shabir G, Rasul G, Khalid MA, Gilani AH. Antioxidant, Antimicrobial Properties and Phenolics of Different Solvent Extracts from Bark, Leaves and Seeds of *Pongamiapinnata* (L.) Pierre. Molecules 2012; 17: 3917-32. http://dx.doi.org/10.3390/molecules17043917

[45] Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QM, Ashrafuzzaman M. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [Delonixregia (Bojer ex Hook.) Raf]. Molecules 2011; 16(9): 7302-19. http://dx.doi.org/10.3390/molecules16097302

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