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Effect of Solvents on the biological activities and phenolic composition of *Achyranthes aspera*

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Abstract

Plants kingdom have a variety of species which are being used for the treatment of various disease in different parts of world including Asia. Synthetic compounds used in the treatment of have side effects and more costly. So it is need to find such compounds from natural sources which have less side effect and economical. So the present research work was focus on the evaluation of *Achyranthes aspera* regarding its antioxidant and antimicrobial potential by extracting in various solvents such as methanol, ethanol and aqueous. Aqueous solvent showed highest extraction yield 10.3% than other solvent. Antibacterial activity was performed by well diffusion assay. Aqueous extract showed maximum antibacterial (29mm and 23mm) activity against both *S. aureous* and *Escherichia coli* respectively by disk inhibition assay. While other both extracts also showed significant activity. Antioxidant activity was performed through 1, 1-diphenyl-2-picrylhydrazyl scavenging free radical assay which showed that all extracts showed significant ($p<0.05$) activity. Methanolic extract showed highest antioxidant activity (68.33%) followed by aqueous extract (53.18%), while ethanolic extract showed lowest antioxidant activity (39.47%). Total phenolic and flavonoid contents were determined by spectrophotometer assay which showed that aqueous extract contain highest phenolic and flavonoid contents as 230 mg/g equ G.A and 137 mg/q equ QE. The characterization of phenolic compounds was done by high performance liquid chromatography which revealed the presence of chromatotropic acid, quercitin, gallic acid, syringic acid, ferulic acid, trans 4-hydroxy-3-methoxy cinamic acid and vinillic acid are the major phenolic in all extracts.

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Introduction

The generation of reactive oxygen species occurs during various metabolic processes in the human body. Free radical is defined as "a molecule or its fragments which contain one or more than unpaired electrons in its outermost molecular or atomic orbital (Ningsih *et al.*, 2016). These species plays a significant role in neurotransmission and cell signaling. In normal tissues, these specifies are also responsible for maintaining the homeostasis (Yamamoto, 2000). Various antioxidant defense system including glutathione (GSH) glutathione peroxidase (GPx) catalase (CAT), superoxide dismutase (SOD) in human body play a role for checking the generation of reactive oxygen species (Fang *et al.*, 2002).

Dietry polyphenol have been getting more attraction due to having potential of inhibition of oxidation of bimolecular through scavenging of free radicals (Urquiaga and Leighton, 2000). It can be achieved by inducing endogenous antioxidant enzymes (La Marca *et al.*, 2012) which can protect human from chronic diseases. Human body has rich sources of antioxidants compounds that have potential to inhibit or limitize the formation of free radicals (Tomas-Barberan and Robinson, 1997; Ningsih *et al.*, 2016).

The currently used antioxidant compounds have effectiveness due to the increases in endogenous free radical scavengers or vitamins (ascorbic acid and alpha tocopherol). Various plants have been founds to have ability to scavenging the free radicals (terpenoids, alkaloids or polyphenols) (Al-Jaber *et al.*, 2011).

Natural products such as plants, herbs and sherbsetc are gaining increases role in scavenging of free radicals and these are now being used for the treatment of various diseases such as diabetes, cancer and heart disorder (Harasym and Oledzki, 2014). Various natural sources including agro wastages, plants, fruits and industrial wastages in form of semisolid form have been studied for the antioxidants extracts that can have biological properties (Fernández-Bolaños *et al.*, 2004).

Now recent study on plants extract, herbs, fruits and vegetables also describes the relationship of antioxidant activities with protection of other biological activities such as anti-carcinogenic disorders, anti-inflammatory analgesic, antiviral, bactericidal (Bakkali *et al.*, 2008). The phytochemical compositions of these natural sources retard the oxidative degradation of lipids and also improve the nutritional value and food quality specially in food industry (Kahkonen *et al.*, 2001) and help in the prevention from microbial activities (Guerrero *et al.*, 2007). The investigation of medicinal plants are required to find their nutritional importance on the basis of biological activities.

Material and methods

Collection of plants sample

The sample of *A. aspera* was collected from district Sargodha, Pakistan. Whole dried plant sample was washed with distilled water for many times for the removal of dust particles and other impurities then grinding to form fine powder and stored in opaque screw-capped containers at room temperature.

Extraction of selected plants by solvents

Powdered material (500g) was taken in a clean, flat bottomed glass container and soaked in methanol, ethanol and aqueous solvents with the ratio of 1:10 plants (sample: solvent). The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45^{o}C) and reduced pressure.

Evaluation of antibacterial activity

Antibacterial activity was performed bu disk inhibition assay against *S. aureous* and *E. coli* bacteria. Nutrient agar (Oxoid) 28 g/L was suspended in distilled water, mixed well and distributed homogeneously. The medium was sterilized by autoclaving at 121˚C for 15 min.

Before the medium was transferred to sterilized Petri plates; inoculation (100 µL/100 mL) was added to the medium and poured in sterilized petriplates. After this, small filter paper discs were laid flat on growth medium containing 100µL of crude methanol extract of crude methanol extract of selected plant.

The petri plates were then incubated at 37˚C for 24 hours, for the growth of bacteria. Crude methanol extract of crude methanol extract of selected plant having antibacterial activity inhibited the bacterial growth and clear zones were formed. The zones of inhibition were measured in millimeters using zone reader (Haung *et al*, 2000).

DPPH scavenging free radicals assay

The antioxidant activity of plantextracts obtained by various solvents was studied by measuring their scavenging ability to 1, 1-diphenyl-2-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by Queiroz *et al*. (2009) with slight modifications. Crudeextract of selected medicinal plants were added at an equal volume in 500µl methanolic solution of DPPH (0.1mM). After 30 minutes incubation at room temperature the absorbance was recorded at 517 nm. The experiment was performed for three times. Ascaric acid was used as a standard. Inhibition of free radical by DPPH was calculated in the following way:

$$I\ (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

Where, A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. Percentage scavenging can be calculated.

Phytochemicals analysis

Total phenolics content

Amount of TPC was calculated using Folin-Ciocalteu reagent as described by Chaovanalikit and Wrolstad (2004). 1 mL of different plant extract concentrationswere taken in a separate test tubes, added 7.5 mL distilled water, then add 0.5 mL Folin-Ciocalteu reagent. Incubate the sample for 90 minutes. Take the absorbance at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid (10-1000 ppm). The results were expressed as Gallic acid equivalents mg GAE/g. Calibration curve is shown in Fig 1.

Total flavonoids content

For the analysis of total flavonoids content aluminium chloride hexahydrate is used as described by Bamane *et al*. (2012) 0.073 g of aluminium chloride hexahydrate is dissolved in 30 mL of methanol. 2 mL of different plants extract concentrations of was taken in seprate test tubes plus add 2 mL of aluminium chloride hexahydrate. Incubate the sample for 15 minutes and measured the absorbance at 430 nm. Quercetin was used as standard flavonoid and the total flavonoid content was calculated as quercetin equivalent antioxidant g/100g dry weight of plant. Amount of TFC was calculated using a calibration curve for Gallic acid (10-1000 ppm). Calibration curve is shown in Fig 2.

Preparation of sample for HPLC analysis (Phenolic Compounds)

The plants extracts for the determination of phenolic compounds were prepared by dissolving 50 mg of each extracts in 24 mL methanol of analytical grade and homogenized the mixture. 16 mL of distilled water was added followed by6M HCl. The mixture was kept in oven at 95°C for 2hours. Then mixture was filtered by using 0.45 um of filter paper and then allowed to analyze on HPLC (Qayyum *et al*. 2016).

Statistical analysis

The obtained results were statistically analyzed by one way analysis of variance by using statistica 8.1 software. The level of significant was set as $P<0.05$.

Results and discussion

Extraction results

Extraction was carried out by using various solvents such as methanol, ethanol and aqueous. Highest extraction yield (10.3%) was shown by aqueous solvent followed by methanol which gives extraction yield (7.8%).

The lowest extraction yield (5.3%) was shown by ethanol as described by Fig 3. There are various factors which affect the extraction yield including selection of solvents, extraction procedure and methodology, extract composition.

These factors may lead to affect the antioxidant potential of plants extract (Brewer, 2011). The phytochemical composition of plants extract is generally eco-friendly, low cost, effectives at low concentrations and nontoxic.

Table 1. Determination of phenolic compound by HPLC analysis.

Compounds	Methanol (ppm)	Ethanol (ppm)	Aqueous (ppm)
Chromatotropic acid	24.5	43.73	-
Quercitin	0.75	10.43	1.34
Gallic acid	6.54	12.5	2.34
Syringic acid	1.2	21.34	-
Ferulic acid	4.56	8.46	-
Trans 4-hydroxy-3-methoxy cinamic acid	0.94	6.34	25.44
Vinillic acid	-	5.65	-
M-coumeric acid	-	2.34	7.54
Caffeic acid	-	-	13.23

Antibacterial Activity

Antibacterial activity of solvent based extracts of *A. aspera* was performed against *S. aureus*and *E. coli*. Highest antibacterial activity was shown by aqueous extract as it inhibit the growth of 29mm of *S. aureous* and 23mm against *E. coli*. methanolic extract showed 23mm inhibiton in growth of *S. aureous* followed by ethanolic extract which inhibit 18mm growth as describe in Fig 4.

In case of inhibition of *E. coli*, ethanolic extract showed 21mm inhibition while methanolic extract showed 19mm inhibition. The results of antibacterial activity were compared with the Rifmpacin which was studied parrallely with this experiment. Rifmpacin showed 37mm inhibition of the both (gram positive and gram negative) bacterial strain which was found highest than the all tested extracts of *A. aspera*.

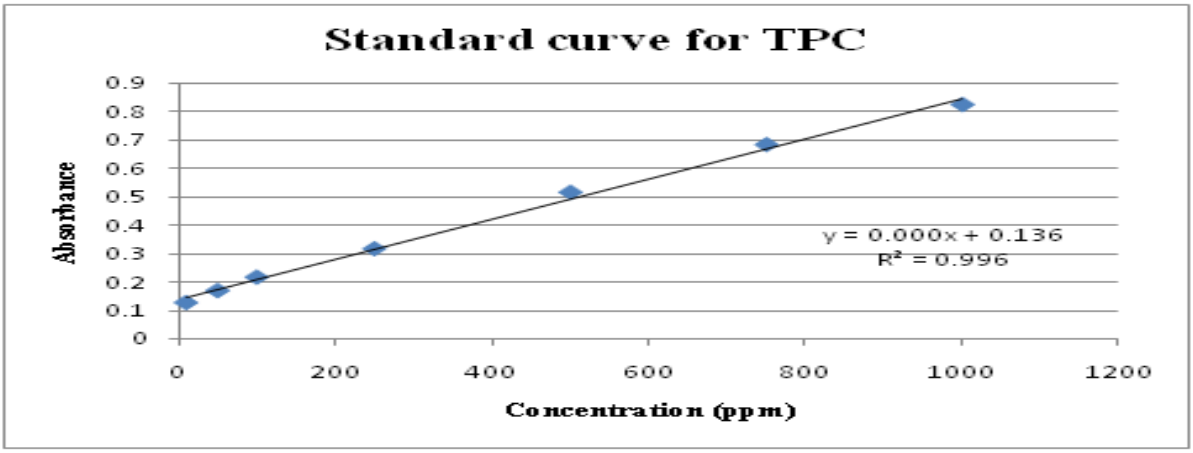

Fig. 1. Calibration curve for TPC.

The results of antibacterial activity were found similar with the antibacterial activity of *P. pungens, N. alba* and *G. Lydia* which showed strong activity (Yildirim *et al*., 2012).

Antioxidant activity

Antioxidant activity of extracts of *A. aspera* was performed by DPPH scavenging free radicals. Highest antioxidant activity 68.33% was shown by methanol

solvent followed by aqueous solvent which showed 53.18% activity as describe in Fig 5. Lowest antioxidant activity 39.47% was shown by ethanol solvent. Ascarbic acid was used as standard which shown highest activity than all the studied extracts. The variation of antioxidant activity is due to variation capacity of solvent to dissolve the various phytochemicals that play an important role in such activity.The results of antioxidant activity was compare with antioxidant activity of methanolic extract of *Syzygium cumini* which showed 70.45% DPPH inhibition (Mohamed *et al.,* 2013). In our results the methanolic extract showed almost similar results.

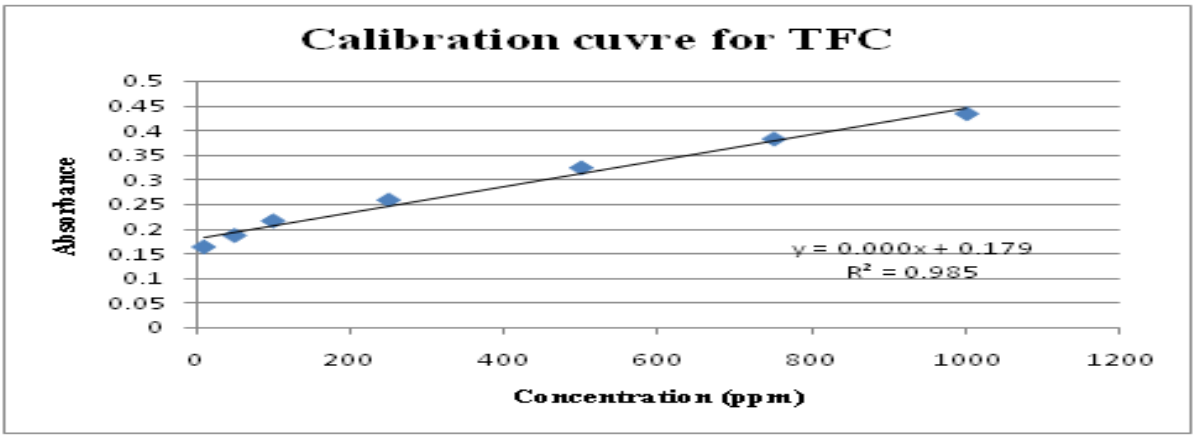

Fig. 2. Calibration curve for TFC.

Total phenolic and flavoinoids contents

Secondary metabolites are the sources of bioactive compounds present in the plants (Bernhoft, 2010). These are present in low quantity in plants and herbs, and their purification, extraction and characterization are performed for drug discovery which is still a great challenge for researchers. The screening of plants is based on the information of the presences of specified secondary metabolites. So for the separation, purification and extraction required an appropriate use of suitable analytical and extraction techniques for best yields.

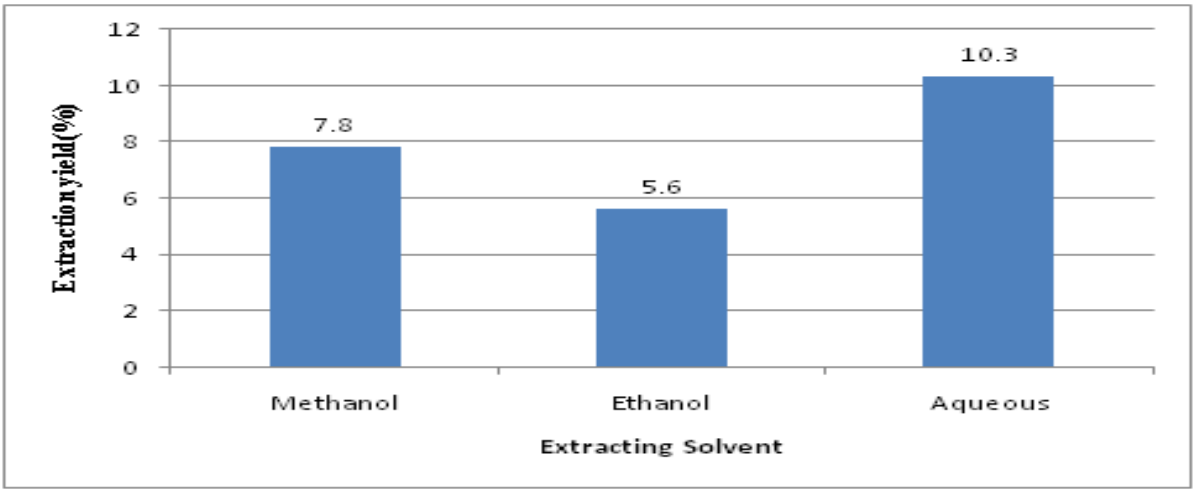

Fig. 3. Extraction yield of *A. aspera*.

Various compounds present in plant extract may contain polyphenols, terpenes, carbohydrates, aldehydes and ketones, esters and alcohols etc.(Singh *et al.*, 2009). Antioxidant approaches have been studied of crude extracts, fractionation and pure compounds. But universal natural antioxidants compounds or agents is still not in used.

So it is necessary to find new compounds or sources for the determination of antioxidants potential. The most reported antioxidant potential is due to phenolic compounds.

Phytochemicals present in plants contain most of phenolic groups which have significant morphological and physiological impact in plants.

The phenolic compounds have a various biological properties due to presence of large group of bioactive compounds. Phenolics compounds can act as pollinators attractive, antifeedants, antioxidants and pigmentation contributors, phytoalexins (Popa *et al.*, 2008), protects the plants pigments from UV light.

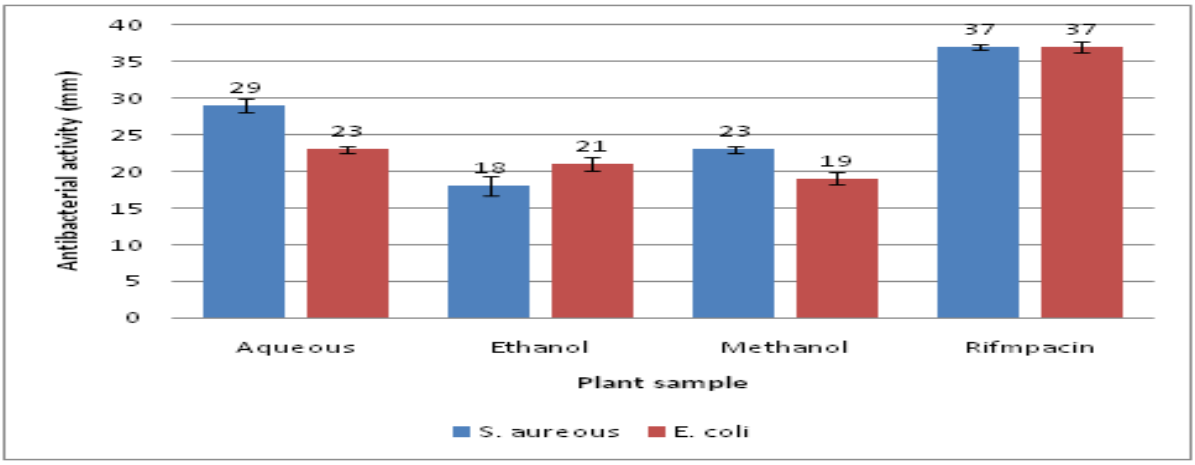

Fig. 4. Antibacterial activity of extracts of *A. aspera*.

Highest phenolic contents (230mg/g equ GA) was shown by aqueous solvents followed by methanolic extract which posses 109mg/g equ GA. The flavonoids contents in aqueous extract found are 137 mg/g equQe while methanolic extract showed 98mg/g equ GAas describe in Fig 6. Ethanolic extract contain lowest contents of both phenolic and flavonoids as it contain phenolic contents 89/g mg/equ GA while flavonoid contents 65mg//g equQe.

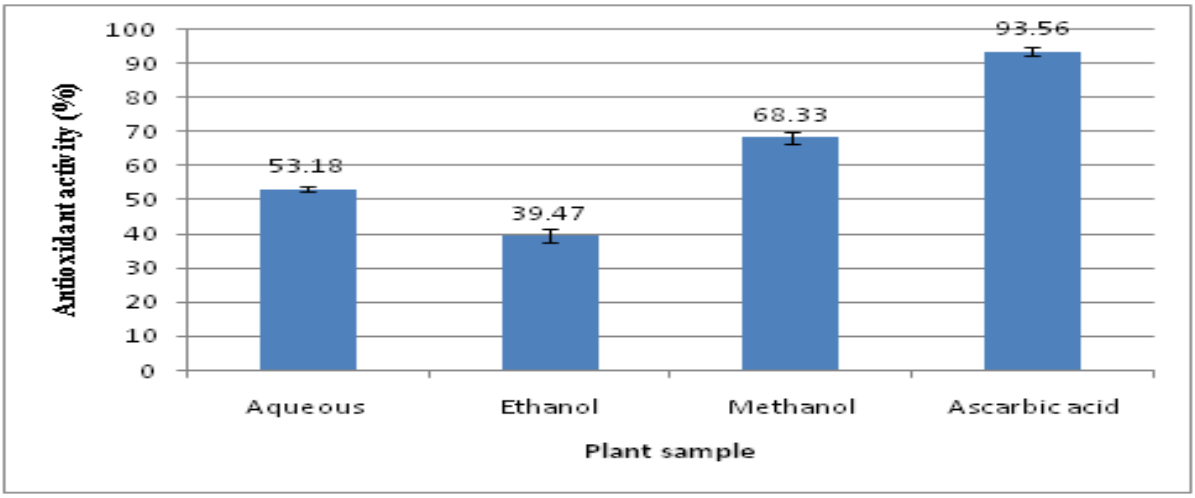

Fig. 5. Antioxidant activity of plant extracts.

Determination of phenolic compounds by HPLC Analysis

Phenolic compounds contain variety of compounds which contain polyphenolic structure such as hydroxyl groups located on aromatic structures. These molecules may also contain one or more phenolic ring structure, for example phenolic and alcohols phenolic acids (Qayyum *et al.* 2016).

Polyphenols are classified in various catagories based on the presence of number of phenolic ring which hold structural elements for binding of one phenolic rings with another. The major classifications polyphenols includes stilbenes and lignans, tannins, phenolic acids and flavonoids (Archivio *et al*., 2007).

The extracts of *A. aspera* was analyzed by reverse phase high performance liquid chromatography for the determination of Quercitin, gallic acid, syringic acid, trans 4-hydroxy-3-methoxy cinamic acid, m-coumeric acid, ferulic acid and Caffeic acid and results were presented in Table 1. Highest chromatotropic acid (43.73 µg/g of dry plant material) was determined in ethanolic extract followed by methanolic extract (24.5 µg/g of dry plant material). Whereas aqueous extract does not contained chromatotropic acid.

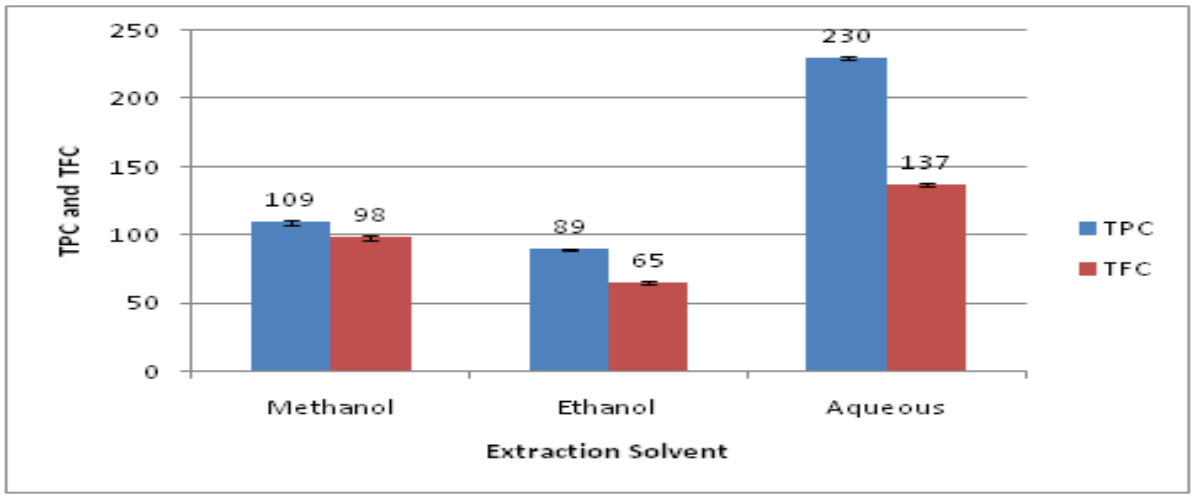

Fig. 6. Determination of total phenolic and flavonoids contents.

The highest quantity of Quercitin (10.43 µg/g of dry plant material) was found in ethanolic contents followed by aqueous extract (1.34 µg/g of dry plant material), while methanolic extract contain (0.75µg/g of dry plant material). Highest amount of gallic acid (12.5µg/g of dry plant material) was found in ethanolic extract followed by methanolic extract (6.54µg/g of dry plant material) while aqueous extract possess least amount (2.34µg/g of dry plant material). Trans 4-hydroxy-3-methoxy cinamic acid was found in highest quantity (25.44 µg/g of dry plant material) followed by ethanolic contents (6.34 µg/g of dry plant material) and methanolic extract (0.94 µg/g of dry plant material). Methanolic and ethanolic extracts also possess the Syringic acid (1.2 µg/g and 21.34 µg/g of dry plant material), ferulic acid (4.56 µg/g and 8.46 µg/g of dry plant material) respectively. Methanolic and aqueous extracts contain m-coumeric(2.34 µg/g and 7.54µg/g of dry plant material). Vinilic acid 5.65µg/g of dry plant material) in ethanolic extract while caffeic acid (13.23µg/g of dry plant material) was found in aqueous extract. The quantitative and qualitative analysis of different extracts was as describe in Table 1.

Conclusion

In present research paper, the effect of solvent for the extraction of phytochemicals which leads to the various biological activities. Antibacterial activity was done against *S. aureous* and *E. coli* bacterial strains by disk inhibition method which showed that aqueous extract showed highest inhibition of both bacterial strains followed by methanolic and ethanolic extract inhibit the growth of *S. aureous* respectively while ethanolic extract have higher inhibition potential against *E. coli* than methanolic extract. Antioxidant activity of different extracts of *A. aspera* was examined by scavenging potential of DDPH free radicals by *in-vitro* assay. Highest antioxidant activity was shown by methanol followed by aqueous solvent. While ethanolic extract showed the least antioxidant activity.

The antioxidant activity was due to presence of various phenolic and flavonoids contents. Aqueous extract possess highest while ethanolic extract showed least total phenolic and flavonoids contents. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Further the characterization of these phenolic compounds was performed by HPLC analysis which revealed the that chromatotropic acid, quercitin, gallic acid, syringic acid, ferulic acid, trans 4-hydroxy-3-methoxy cinamic acid, vinillic acid, m-coumeric acid and caffeic acid are phytochemical compounds which are the responsible of such activity. Due to multifunctional properties, antimicrobial potential and purified from natural sources like animals, plants and microorganism etc. are being considered as alternative to synthetic antibiotics against which most of the infection causing agents have developed resistance.

Reference

Al-Jaber NA, Awaad AS, Moses JE. 2011. Review on some antioxidant plants growing in Arab world. Journal of Saudi Chemical Society, **15**, 293-307. http://dx.doi.org/10.1016/j.jscs.2011.07.004

Archivio DM, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. 2007. Polyphenols, dietary sources and bioavailability. Annali-IstitutoSuperiore di Sanita, **43**, 348-352.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils–a review. Food and chemical toxicology, **46**, 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106

Bamane FH, Badr JM, Amin OARM. 2012. Antioxidant activities and flavonoid contents of selected plants belonging to Family Loranthaceae. African Journal of Biotechnology, **11**, 14380-14385. http://dx.doi.org/10.5897/AJB12.2093

Bernhoft A. 2010. A brief review on bioactive compounds in plants. Bioactive compounds in plants-benefits and risks for man and animals.

Brewer MS. 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive Reviews in Food Science and Food Safety **10**, 221-247. http://dx.doi.org/10.1111/j.15414337.2011.00156.x

Chaovanalikit A, Wrolstad RE. 2004. Anthocyanin and polyphenolic composition of fresh and processed cherries. Journal of Food Science, **69**, FCT73-FCT83. http://dx.doi.org/10.1111/j.1365-2621.2004.tb17859.x

Fang YZ, Yang S, Wu G. 2002. Free radicals, antioxidants, and nutrition. Nutrition, **18,** 872-879. http://dx.doi.org/10.1016/S08999007(02)00916-4

Fernández-Bolaños J, Rodríguez G, Gómez E, Guillén R, Jiménez A, Heredia A, Rodríguez R. 2004. Total recovery of the waste of two-phase olive oil processing: isolation of added-value compounds. Journal of agricultural and food chemistry **52,** 5849-5855. http://dx.doi.org/10.1021/jf030821y

Guerrero-Rodríguez E, Solís-Gaona S, Hernández-Castillo FD, Flores-Olivas A, Sandoval-López V, Jasso-Cantú D. 2007. Actividad Biológica *in vitro* de Extractos de Flourensiacernua DC en Patógenos de Postcosecha: *Alternaria alternata* (Fr.) Keissl., *Colletotrichum gloeosporioides* (Penz.) Penz. Sacc., *Penicillium digitatum* (Pers.: Fr.) Sacc. *Revista mexicana* de Fitopatología **25,** 48-53.

Harasym J, Oledzki R. 2014. Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. Nutrition **30,** 511-517. http://dx.doi.org/10.1016/j.nut.2013.08.019

Huang D, Ou B, Prior RL. 2005. The chemistry behind antioxidant capacity assays. Journal of agricultural and food chemistry, **53,** 1841-1856. http://dx.doi.org/10.1021/jf030723c

Kähkönen MP, Hopia AI, Heinonen M. 2001. Berry phenolics and their antioxidant activity. Journal of Agricultural and Food Chemistry,**49**, 4076-4082. http://dx.doi.org/10.1021/jf010152t

La Marca M, Beffy P, Della Croce C, Gervasi PG, Iori R, Puccinelli E, Longo V. 2012. Structural influence of isothiocyanates on expression of cytochrome P450, phase II enzymes, and activation of Nrf2 in primary rat hepatocytes. Food and chemical toxicology **50,** 2822-2830. http://dx.doi.org/10.1016/j.fct.2012.05.044

Mohamed A A, Sami IA, El-Baz FK. 2013. Antioxidant and Antibacterial Activities of Crude Extracts and Essential Oils of *Syzygium cumini* Leaves. PLoSONE **8,** 6026-6029. http://dx.doi.org/10.1371/journal.pone.0060269

Ningsih IY, Zulaikhah S, Hidayat MA, Kuswandi B. 2016. Antioxidant Activity of Various Kenitu (*Chrysophyllum cainito* L.) Leaves Extracts from Jember, Indonesia. Agriculture and Agricultural Science Procedia **9,** 378-385. http://dx.doi.org/10.1016/j.aaspro.2016.02.153

Popa VI, Agache C, Beleca C, Popa M. 2002. Polyphenols from spruce bark as plant growth regulator. Crop Research-Hisar **24,** 398-406.

Qayyum A, Sarfraz RA, Ashraf A, Adil S. 2016. Phenolic composition and biological (anti diabetic and antioxidant) activities of different solvent extracts of an endemic plant (*Heliotropium strigosum*). Journal of the Chilean Chemical Society, **61,** 2828-2831. http://dx.doi.org/10.4067/S07179707201600010016

Queiroz YS, Ishimoto EY, Bastos DHM, Sampaio GR, Torres, EAFS. 2009. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: *In vitro* antioxidant activity. Food Chemistry, **115,** 371–374. http://dx.doi.org/10.1016/j.foodchem.2008.11.105

Singh HP, Mittal S, Kaur S, Batish DR, Kohli RK. 2009. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of Eucalyptus tereticornis. Journal of agricultural and food chemistry **57,** 6962-6966. http://dx.doi.org/10.1021/jf9012407

Tomás-Barberán FA, Robins RJ. 1997. Phytochemistry of fruit and vegetables. In International Symposium of Phytochemistry of Fruit and Vegetables (1995: Murcia, Spain). Clarendon Press; Oxford University Press.

Urquiaga INES, Leighton F. 2000. Plant polyphenol antioxidants and oxidative stress. Biological research, **33**, 55-64. http://dx.doi.org/10.4067/S07169760200000020004

Yamamoto YY. 2000. Free radicals in chemistry, biology and medicine. OICA International (UK).

Yildirim AB, Karakas FP, Turker AU. 2012. In vitro antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey. Asian Pacific Journal of Tropical Medicine **6**, 616-624