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ANTIOXIDANT ACTIVITY OF RADISH LEAVES EXTRACTS PRODUCED BY MICROWAVE ASSISTED AND CONVENTIONAL EXTRACTION METHODS

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Abstract –This study focused on antioxidant activity of radish leaves extract produced by Microwave Assisted Extraction (MAE), Soxhlet Extraction (SE) and Maceration with different ethanol (EtOH) concentrations. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were analyzed by Folin-ciocaltteu method and Aluminium Chloride method respectively. Free radical scavenging activity was evaluated by Diphenyl Picryl Hydrazyl (DPPH) method and Half-maximal inhibitory concentration (IC50) value was recoded. The maximum total phenolic and total flavonoids were found as 177.5 mg/g gallic acid equivalent (GAE) and 55.09 mg/g Quercetin equivalent (QE) respectively, using 90% EtOH concentration with microwave at 300W. 90% ethanolic radish leaves extracts showed the highest free radical scavenging activity with power of 400W of 78.70% with an IC 50 value of 65.73 µg/ml. In all of assay, 90% EtOH Radish leaves extracts with MAE showed higher phytochemicals and antioxidant activity, suggesting that MAE is a suitable method for extraction of antioxidant component from radish leaves and may be investigated as potential raw materials for creation of nutritional supplements.

INTRODUCTION

Human health conditions caused by oxidative stress are now a major concern. According to the World Health Organization (WHO), traditional medicine is used by 80% of the world's population for their primary healthcare requirements. The majority of this therapy uses plant extracts and their active ingredients (Krishnaiah *et al.*, 2011). Natural antioxidants are mostly found in plants, and because they can guard against the effects of free radicals, the potential of these bioactive constituents has been thoroughly researched.

The root vegetable radish (*Raphanus sativus* L.), which belongs to the Cruciferaceae family, is a significant vegetable crop grown around the world (Tsouvaltzis and Brecht, 2014). The composition of radish was discovered to provide significant therapeutic and dietary benefits. As a result, it was proposed as a complementary therapy for a number of conditions, such as cancer, hyperlipidemia, and coronary heart disease (Curtis, 2003). Herbal medicines uses various parts of the plant, including the leaves, seeds, and roots, to treat gastrointestinal disorders as well as biliary, liver, urinary, respiratory, and cardiovascular conditions (Devraj, *et al*., 2011; Shin *et al*., 2015). Additionally, the plant has anti-inflammatory bioactives (Park and Song, 2017; Manivannan *et al*., 2019).

Factors including the extraction process, the solvent used for extraction, and the solvent ratio all affect the quality of the extracts and the amount of active chemicals they contain. Therefore, it would be important to optimise the extraction processes in order to achieve high extraction efficiency for medicinal plants. In addition to traditional approaches, there are a few novel extraction methods for medicinal plants. Traditional techniques include maceration, percolation, thermal digestion, and soxhlet, while contemporary techniques include microwave assisted extraction (MAE), Ultrasonication Assisted Extraction (UAE), Super critical Fluid Extraction (SFE), Solid Phase Micro Extraction (SPME) and etc. (Sharifi *et al.*,

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2017). The aim of this study was to prepare extracts from radish leaves by using MAE and conventional (soxhlet and maceration) methods and test their antioxidant activity and phytochemical analysis.

MATERIALS AND METHODS

The experiment was carried out in the laboratory of Department of Processing and Food Engineering, Vaugh Institute of Agricultural Engineering and Technology, SHUATS, Prayagraj, India.

Materials

Radish leaves were purchased from a nearby vegetable vendor at Gangotri Nagar, Prayagraj. Clean leaves were dried in tray dryer at 50 to 60 °C and crushed in grinder. Powdered radish leaves were stored in polyethylene pouches at 4°C in refrigerator for further study.

Ethanol, Methnol, Ascorbic acid, Querctin, Gallic acid, Sodium Carbonate, Aluminium Chloride, Potassium Acetate, DPPH, Folin-ciocaltue reagents were used for various extraction and analysis purpose. All chemicals used were of analytical grade.

Extraction Procedure

The hot air oven method was used to determine the moisture content of the powdered radish leaves, which was 6.6 % on a dry basis prior to extraction. Leaf extracts of radish were made using three distinct extraction techniques i.e. MAE, Soxhlet and Maceration. Extracts were prepared using three different ethanol concentrations: 30%, 60%, 90%.

Microwave Assisted Extraction

In the conical flask, 15 g of powdered sample were weighed and 100 ml of solvent was added.The solution was then thoroughly blended using a stirrer. The flask was put in the microwave and exposed to radiation for 5 min at 200W, 300W, and 400W of power. The extract was filtered and dried in a water bath at 50°C after cooling. The resulting dried residue was kept at 4°C till further use.

Soxhlet Extraction

Fifteen grams of radish leaves powder were placed in a filter paper thimble and kept in the extractor unit of the Soxhlet apparatus. 100 ml of ethanol at various concentrations were used to extract the sample for 8 h. The extract was dried in a water bath at 50°C after cooling. The resulting dried residue was kept at 4°C till further use.

Maceration Extraction

At room temperature, 100 ml of solvents were added to 15 g of sample to soak for 24 h. A strainer was used to remove coarse particles. Later, filter paper was used to extract the residue once more. The extracted material was dried at 50°C in a hot air oven and water bath. All dried extract yields were estimated using the formula described in equation 1.

$$\% \text{ Extraction yield} = \frac{\text{Dried wt. of extract}}{\text{Total wt. of skin power}} \times 100 \qquad \text{Eq. 1}$$

Determination of Total Phenolic Content

The Total Phenolic of the extract was determined using the Folin and Ciocalteu reagent method described by Chandra *et al.* (2014) with slight modifications. Using a spectrophotometer (Make: Microtech Venus, Model: vis002) at 765 nm in comparison to the reagent blank, sample and standard readings were taken. All determinations were made in triplicate.

Determination of Total Flavonoid Content

Total Flavonoid Content of extract was estimated spectrophotometrically according to Aluminium Chloride method described by Kalra *et al.,* (2016). 1.9 ml of methanol was mixed in 0.1 ml of extract of radish leaves. 0.1 ml of 10% Aluminium Chloride and 0.1 ml of 1 M Potassium Acetate was combined in extract respectively and volume was made up to 5 ml with distilled water. The above solution was then allowed to stand at room temperature in dark and the absorbance of the above mixture was calculated at 415 nm. Every determination was conducted in triplicate.

Antioxidant Activity

The DPPH method was used to assess the antioxidant activity of radish leaves. The DPPH solution (.01 mM) was obtained by dissolving 4 mg DPPH in 100 ml methanol. Various concentrations of extracts (20-200 µg/ml) were mixed with 3 ml of DPPH solution. The mixture was thoroughly mixed and kept in dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer against a methanol blank with DPPH, excluding extract. The antioxidant activity or free radical scavenging activity was calculated using the formula shown in equation 2.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad \text{Eq. 2}$$

The half maximum inhibitory concentration (IC50) was defined as the amount of antioxidant needed to decrease the initial DPPH concentration by 50%.

Statistical Analysis

Experiments were conducted in triplicate. Data were expressed as the means of these values ± the standard deviations (SD). Analysis of variance (ANOVA)-one way ANOVA was used to analyze data for knowing its significance with respect to 5% probability level.

RESULTS AND DISCUSSION

The raw vegetables' volatile component concentration has the significant positive correlation with the antioxidant activity of crude extracts. Since the non-polarity of water and the polar character of the majority of phenolic compounds, it is frequently required to change the solvent power of the fluid during extractions in order to increase the yield (Pereira *et al.*, 2013). Because it is an environmental friendly and generally recognized as safe (GRAS) solvent, ethanol can be utilised in fruit and vegetables extraction procedures (Solana *et al.*, 2014,) In current investigation ethanol was used as solvent, with different concentrations. Extraction yield of radish leaves crude extracts were shown in Table 1 with various concentration of ethanol. Microwave technique exhibited higher extraction yield (35.33 %) with 30% ethanol solvent at 400W and soxhlet showed lower yield of 14.44 % with 90% ethanol. Extraction yield of leaves was comparable with Silva *et al.,* (2020) for the same extract of 30.40% but lower compared to the value of 25.60% as reported by Senguttuvam *et al*. (2014). It was observed that the overall extract yield shown to slowly decline till the ethanol concentration reached 90%. Appropriate swelling of the vegetable matter for improved extraction yield requires a considerable amount of water (Veggi *et al.*, 2013).

Total Phenolic content were estimated with the help of calibration curve of Gallic acid (50 to 500 mg/g), shown in Fig. 1. TPC of different radish leaves

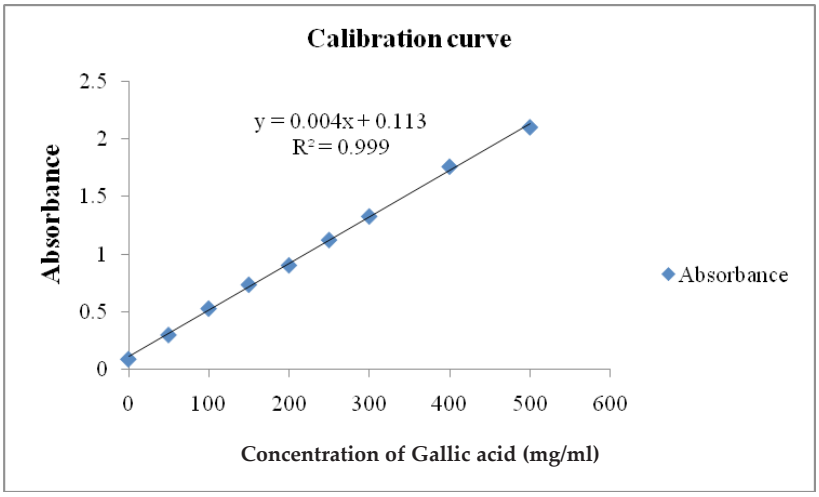

Fig. 1. Calibration curve for Total Phenolic Content

Table 1. Extraction yield of radish leaves extract using different methods with different concentration of ethanol (EtOH)

EtOH conc.	MAE[a] (yield in %)			SE[b]	M[c]
	200W	300W	400W	60 °C	Room temp.
30% EtOH	29.83± 0.23	33.33± 1.33	35.33± 1.91	25.81± 1.73	28.31± 0.41
60% EtOH	24.19± 4.16	30.84± 1.47	31.10± 0.95	22.65± 2.31	24.88± 1.47
90% EtOH	24.60± 1.19	27.57± 3.34	30.11± 1.29	14.44± 2.54	18.04± 4.88

Data were represented as the mean value ± standard deviation
a. Microwave Assisted Extraction
b. Soxhlet Extraction
c. Maceration

extracts varied greatly, ranging from 177.5 to 46.58 mg GAE/g extract which was shown in Table 2. Extracts from microwave shows higher TPC than that of soxhlet and maceration. Routray and Orsat, (2014) showed similar results of 131 mg GAE/g for blueberry leaves with 30% ethanol solution.Given the quick and effective separation of the bio-cellular component with the right polar solvents, leading to significant extraction efficiency, the combination of solvents with chosen microwave parameters, which were power level and time of microwave selected, promotes it as a promising technique of extracting TPC from organic material.

Total flavonoid content was presented in Table 3, which was varied from 6.12 (maceration with 30% EOH) to 55.09 mg QE/g (MAE at 300W with 90% EOH). All the higher concentration of ethanol extracts showed maximum TFC, due to polar nature of flavonoids and TFC estimation was done by the calibration curve of Quercetin (10 to 100 mg/g), which was shown in Fig. 2. TFC was analyzed by Saeed *et al*., (2012) for *Torilisleptophylla* leaves by using various fractions of solvent and comparable with the results obtained.

A distinct category of phenolic compounds known as flavonoids has a framework based on the

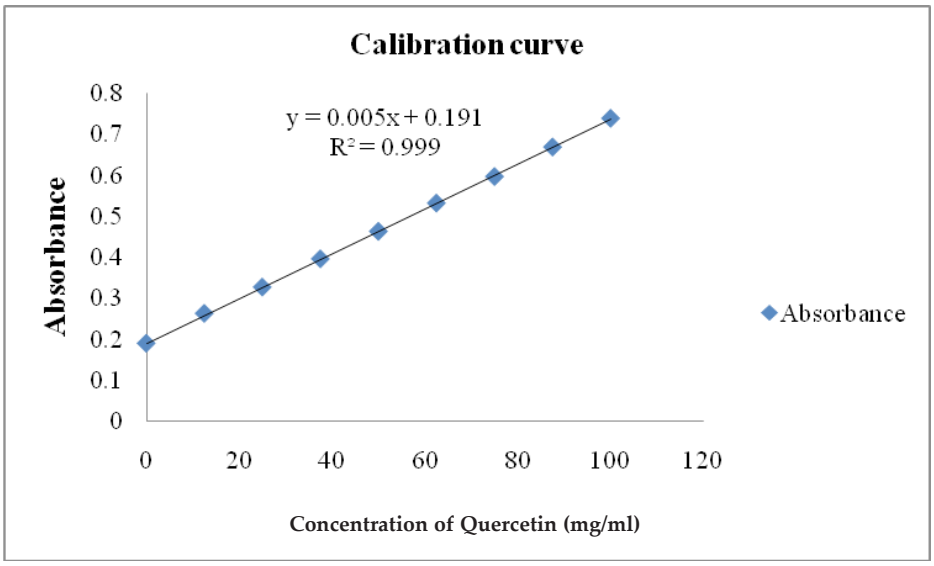

Fig. 2. Calibration curve for Total Flavonoid Content

Table 2. Total Phenolic content of radish leaves extract using different methods with different concentration of ethanol (EtOH)

EtOH conc.	MAE[a] (TPC in mg GAE/g)			SE[b]	M[c]
	200 W	300 W	400 W	60 °C	Room temp.
30% EtOH	97.66 ± 0.62	143.8 ± 3.97	99.83 ± 0.38	49.83 ± 0.62	46.58 ± 0.62
60% EtOH	100.5 ± 0.38	156.3 ± 0.28	105.6 ± 4.40	58.6 ± 0.28	56.5 ± 0.75
90% EtOH	108.9 ± 0.28	177.5 ± 0.25	115.1 ± 2.52	92.91 ± 0.62	59.08 ± 0.62

Data were represented as the mean value ± standard deviation
a. Microwave Assisted Extraction
b. Soxhlet Extraction
c. Maceration

Table 3. TFC of radish leaves extract using different methods with different concentration of ethanol (EtOH)

EtOH conc.	MAE (TFC in mg QE/g)			SE	M
	200 W	300 W	400 W	60 °C	Room temp.
30% EtOH	16.18±0.62	22.12±3.97	18.79±0.38	12.61±0.62	6.12±0.62
60% EtOH	24.67±0.38	31.82±0.28	28.97±4.40	11.27±0.28	6.30±0.75
90% EtOH	44.±0.28	55.09±0.25	50.43±2.52	36.73±0.62	35.33±0.62

carbon skeleton of diphenyl propane. Typically, flavonoids have more hydroxyl groups and have stronger antioxidant properties (Kim *et al.,* 2013).

It is well recognised that free radicals play a significant part in a wide range of clinical symptoms. Antioxidants save us from numerous diseases by battling free radicals. They either work by removing singlet oxygen from the environment or by defending the antioxidant defence systems (Umamaheswari and Chatterjee, 2008). DPPH method was used for estimation of antioxidant activity through which IC50 value was recorded, which was showed in Fig. 3.

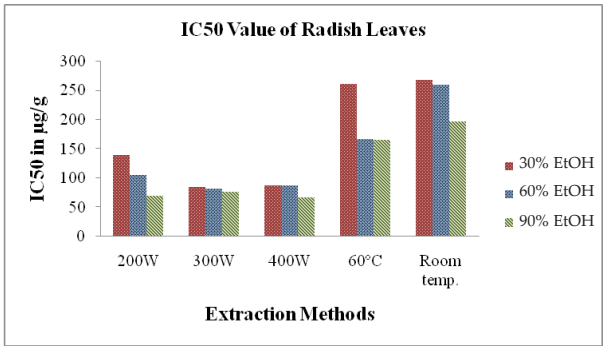

Fig. 3. IC50 values for radish leaves extract using different methods with various ethanol conc.

Radish leaves extract with microwave showed maximum antioxidant activity than that of maceration and soxhlet and IC_{50} value for microwave was lower (65.7 µg/ml) in comparison with soxhlet (260.3 µg/ml) and maceration (267.6 µg/g). IC50 value is inversely proportional to the antioxidant activity. Eugenio *et al.* (2017) found higher value of 422.7 µg/ml for methanolic extract of radish leaves, which was higher than obtained results. In previous experiment methanol was used as solvent which shows lower antioxidant activity than the obtained results. This can be described by a number of variables, such as the presence of various chemical components in the leaves, the type of plant, the extraction solvent, which can alter the antioxidant activity due to the synergistic effects of various compounds, the operational conditions, and the mechanisms of the methods used to assess the antioxidant activity (Brum *et al.,* 2013 and Jayaprakasha and Patil, 2007).

CONCLUSION

In the present work, antioxidant activity of radish leaves extracts could be explained by the presence of phenolics and flavonoids. Extracts which consist of high phytochemicals exhibited higher antioxidant activity and extracts prepared by microwave have higher antioxidant activity. Research suggests that radish leaves may be used as a source of bioactive substances. Additionally, it is important to maximize the extraction of such chemicals from leaves in order to preserve food organically without the need of artificial additives, hence promoting the creation of new useful products.

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Conflict of Interest: None

REFERENCES

Brum, T.F., Zadra, M., Piana, M., Boligon, A.A., Frohlich, J.K., Freitas, R.B.F., Stefanello, S.T., Froeder, A.L.F., Belke, B.V., Nunes, L.T., Jesus, R.S., Machado, M.M., Rocha, J.B.T., Felix Soares, A.A. and Athayde, M.L. 2013. HPLC Analysis of Phenolics Compounds and Antioxidant Capacity of Leaves of *Vitexmegapotamica* (Sprengel) Moldenke. *Molecules*. 18 : 8342-8357.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M.H., ElSohly, M.A. and Khan, I.A. 2014. Assessment of Total Phenolic and Flavonoid Content, Antioxidant Properties, and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. *Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine* Vol. 2014, Article ID 253875.

Curtis, I.S. 2003. The noble radish: Past, present and future. *Trends in Plant Science*. 8(7): 305-307.

Devraj, V.C., Krishna, B.G. and Viswanatha, G.L. 2011. Simultaneous determination of quercetin, rutin and kaempferol in the leaf extracts of *Moringaoleifera* Lam. and *Raphanus sativus*Linn. by liquid chromatography-tandem mass spectrometry. *Chinese Journal of Integrative Medicine*. 19(3) : 1022-1030.

Eugenio, M.H.A., Pereira, R.G.F.A., Abreu, W.C. and Pereira, M.C.A. 2017. Phenolic compounds and antioxidant activity of tuberous root leaves. *International Journal of Food Properties*. 20(12): 2966-2973.

Jayaprakasha, G.K. and Patil, B.S. 2007. In Vitro Evaluation of the Antioxidant Activities in Fruit Extracts from Citron and Blood Orange. *Food Chemistry*. 101: 410–418.

Kalra, S., Midha, K. and Kaur, S. 2016. Purification of quercetin by HPLC from green tea leaves and its

application in cancer therapy. *Indian Journal of Research in Pharmacy and Biotechnology.* 4(2): 2320-3471.

Kim, J. S., Kang, O. J. and Gweon, O. C. 2013. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. *Journal of Functional Foods*. 5(1) : 80-86.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. 2011. A review of the antioxidant potential of medicinal plant species. *Foodand Bioproducts Processing*. 89(3): 217–233.

Manivannan, A., Kim, J., Kim, D., Lee, E. and Lee, H. 2019. Deciphering the Nutraceutical Potential of *Raphanus sativus*-A Comprehensive Overview. *Nutrients*. 11(402) : 1-15.

Moura, P.M., Prado, G.H.C., Meireles, M.A.A. and Pereira, C.G. 2012. Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data. *The Journal of Supercritical Fluids*. 62: 116-122.

Park, H. J. and Song, M. 2017. Leaves of *Raphanus sativus* L. Shows Anti-Inflammatory Activity in LPS-Stimulated Macrophages via Suppression of COX-2 and iNOS Expression. *Preventive Nutrition and Food Science*. 22(1) : 50-55.

Pereira, P., Bernardo-Gil, M.G., Cebola, M.J., Mauricio, E. and Romano, A. 2013. Supercritical fluid extracts with antioxidant and antimicrobial activities from myrtle (*Myrtus communis* L.) leaves. Response surface optimization. *The Journal of Supercritical Fluids*. 83: 57-64.

Routray, W. and Orsat V. 2014. MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Industrial Crops and Products*. 58 : 36-45.

Saeed, N., Khan, M.R. and Shabbir, M. 2012. Antioxidant activity, total phenolic and flavonoid contents of whole plant extracts *Torilislep tophylla* L. *BMC Complementary and Alternative Medicine*. 12: 221

Senguttuvan, J., Paulsamy, S. and Karthika, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*. 4(1): 359-367.

Sharifi, N., Mahernia,S., Amanlou, M. 2017.Comparison of Different Methods in Quercetin Extraction from Leaves of *Raphanus sativus* L. *Pharmaceutical Sciences.* 23: 59-65.

Shimada, K., Fujikawa, K., Yahara K. and Nakamura, T.1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextinemultion. *Journal of Agri. Food Chem*. 40 : 945-948.

Shin, T., Ahn, M., Kim, G.O. and Park, S.U. 2015. Biological activity of various radish species. *Oriental Pharmacy and Experimental Medicine*. 15(2): 105-111.

Silva, A.F., Lopes, M.O., Cerdeira, C.D., Ribeiro, I.S., Rosa, I.A., Jorge Kleber Chavasco, J.K., Silva, M.A., Marques, M.J. and Silva, G.A. 2020. Study and evaluation of antimicrobial activity and antioxidant capacity of dry extract and fractions of leaves of *Raphanus sativus* var. *oleiferus* Metzg. *Bioscience Journal*. 36(2): 606-618.

Solana, M., Boschiero, I., Dall'Acqua, S. and Bertucco, A. 2014. Extraction of bioactive enriched fractions from *Eruca sativa* leaves by supercritical CO2 technology using different co-solvents. *The Journal of Supercritical Fluids*. 94: 245-251.

Tsouvaltzis, P. and Brecht, J. K. 2014. Changes in quality and antioxidant enzyme activities of bunched and topped radish (*Raphanus sativus* L.) plants during storage at 5 or 10 °C. *Journal of Food Quality*. 157-167.

Umamaheswari, M. and Chatterjee, T.K. 2008. In vitro antioxidant activities of the fractions of *Coccinniagrandis* L. leaf extract. *Afr J Trad Compl Altern Med*. 5 : 61-73.

Veggi, P.C., Martinez, J. and Meireles, M.A.A. 2013. Fundamentals of Microwave Extraction, in Microwave-Assisted Extraction for Bioactive Compounds: Theory and Practice, vol. 4, *Food Engineering series*, ed. by F. Chem, G. Cravotto (Springer Science & Business Media, New York, 2013), page 15.