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Qualitative and Quantitative RP-HPLC-PDA Method of Analysis of Polyphenols in Lyophilized Wheat Seedling Juice Powder

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ABSTRACT: Wheatgrass has anti-oxidant, anti-cancerous and anti-ulcerative potential due to the presence of adequate amount of chlorophyll, vitamins, enzymes and secondary metabolites like phenols, flavonoids and alkaloids. Most of the phytoconstituents has been found in methanol and water extracts of wheat seedling juice powder that analyzed on qualitative basis by following some chemical tests. Phenols and flavonoids also determined on quantitative basis by considering calibration curves of standard gallic acid and rutin. Highest phenolic content i.e., 56.67 ± 5.77 GAE mg/g determined in methanolic extract and higher amount of flavonoid content analyzed in ethyl acetate extract (0.313 ± 0.058 RE mg/g). Some of the important polyphenols quantified by RP-HPLC-PDA method in wheat seedling juice powder extract in which epicatechin (2.42µg/mL) determined in higher amount and cinnamic acid (0.43µg/mL) in lesser amount. Reversed phase-high performance liquid chromatography recognized as most précised and advanced technique to analyze polyphenols that overcome the limitations of other methods. Nine polyphenols determined through RP-HPLC-PDA method in wheat seedling juice powder.

Keywords: Wheat seedlings, phenols, flavonoids, anti-oxidant, anti-cancer, RP-HPLC-PDA.

I. INTRODUCTION

Wheatgrass carried lots of essential and non-essential amino acids, chlorophyll content, vitamins like A, C, E, B-complex, B12 and contained multiple minerals such as calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron and molybdenum [1, 2]. In addition to nutritional values wheatgrass has much pharmacological diversity due to the presence of phenols, flavonoids, hydrocarbons, saponins, triterpenes, phytosterols, alkaloids, tannins, glycosides and proteinogenic compounds. Some indole compounds like amygdalin (vitamin B17), choline and the glycoside molecules have potentiality to impede DNA oxidative damage i.e., isolated from wheat seedlings [3, 4]. Phenolic compounds known as secondary metabolites formulated during plant growth period against stress conditions which played pivot role in combating oxidative stress in the human body by maintaining a balance between oxidants and anti-oxidants [5, 6]. The existence of phenolic compounds recognized in free soluble form and bound insoluble forms in cereal grains. The qualitative and quantitative analysis of these compounds accomplished by reversed phase-high performance liquid chromatography (RP-HPLC) [7]. The major phenolic components such as gallic acid, ellagic acid, syringic acid, benzoic acid, quercetin (plant flavonoid), butylated hydroxyl anisole and hydroxylcinnamic acids like caffeic acid, ferulic acid, p-coumaric acid have been identified in wheatgrass extracts [8, 9]. Phenolic compounds have wide range of physiological properties like anti-coagulant, dilation of blood vessels, reduced myocardial dysfunction, artherogenic inhibition, minimized allergic reactions, intermediates for biosynthesis of secondary metabolites, retard the growth of malignant cells and slow down the growth of pathogenic microorganisms and reduced cell damage caused due to unstable particles [10, 11]. Bioflavonoids, plant derived compounds used in alternative medicine as an assistance to intensify anti-oxidant and anti-cancerous potential. Some of the bioflavonoids like apigenin, quercetin, luteonin have been identified in wheatgrass. Quercetin and luteonin, polyphenolic flavonoid have potential to revert carcinogenesis. Apigenin, a bioactive flavonoid procured from wheat sprouts used as an anti-neoplastic and inflammation reducing agent. Agropyrene have anti-inflammatory properties and indole helped in enzyme synthesis and carcinogen deactivation in the liver [12]. Flavonoids ceased lipid peroxidation that responsible for several illnesses like arteriosclerosis, hepatic toxicity, diabetes and inflammation which enhanced along aging [13, 14]. Wheatgrass has ability to scavenge the free radicals and protect the human body from oxidative stress and it's used by many Ayurveda practitioners since ages because of the presence of high level phenolic and flavonoid content enriched with anti-oxidant properties. The phenolic and flavonoid compounds of wheatgrass have played important role in membrane protection by scavenging free hydroxyl, peroxyl radicals and quenched formation of ferrozine-Fe^{2+}complex [15, 16].

II. MATERIALS AND METHODS

A. Materials and Chemicals

Surface sterilized seeds of wheat (*Triticum aestivum* L.) have been taken to grow wheatgrass at lab scale. At

jointing stage wheat seedling harvested and extracted juice proceeded for lyophilization. Lyophilized juice powder further used for formulation of different extracts like hexane, ethyl acetate, methanol and water by using rotary evaporator. Salts of Hi Media and analytical grade solvents were used for experimentation.

B. Qualitative Phytochemical Analysis

The qualitative based phytochemical analysis of lyophilized WSJP (Wheat Seedling Juice Powder) extracts was done by different methods [17-19].

Test for Carbohydrates: 1mL of Molisch reagent and 3-4 drops of concentrated H_2SO_4 were added in 2mL of each extracts. The reddish colored appearance was showed the carbohydrate presence.

Test for Reducing Sugars: In 2mL of each extracts, 5mL of Fehling's solution A and B (1:1) was added and put in boiling water bath for 10 minutes. Appearance of brick red precipitation showed the availability of reducing sugars.

Test for Proteins: Took 2mL of each extracts, add 4-5 drops of Ninhydrin (0.2%) in it and put into boiling water bath for 25 minutes. The appearance of bluish color was showed the availability of proteins.

Test for the Presence of Anthraquinones: The 10mL benzene was added to 0.5g of each extracts and shaken vigorously. Then filtered the reaction mixture with Whatman filter paper no. 1 and add 5mL of 10% ammonia solution to the filtrate. The reaction mixture was mixed by shaking and the presence of a pinkish red color in the lower phase showed the anthraquinones presence.

Test for Saponins: The 1mL of each extracts was taken in test tube with 8-10mL distilled water and shake vigorously. After shaking left the extract at room temperature for 8-10 minutes and then formation of froth indicated the presence of saponins. Addition of few olive oil drops formed the emulsion.

Test for Glycosides: The 2mL of each extracts was mixed with glacial acetic acid (1mL) and 5% $FeCl_3$ chloride with 3-4 drops of concentrated H_2SO_4. The formation of greenish blue coloration showed the availability of glycosides.

Test for Phenols: The 2mL of distilled water added into 1mL of each extracts with 5-6 drops of ferric chloride (10%). The appearance of bluish green color showed the presence of phenols.

Test for Flavonoids: The 2mL of each extracts was reacted with concentrated H_2SO_4 (4-5 drops) and the formation of orange color showed the availability of flavonoids.

Test for Tannins: In 2mL of each extracts, 4mL of 5% $FeCl_3$ was added. The appearance of bluish color showed the presence of tannins.

Test for Fixed Oils: The 2-3 drops of each extracts were passed through Whatman filter paper number 1. The availability of fixed oils was specified by the appearance of oil strain.

Detection of Coumarins: In 5mL of each extracts, few drops of alcoholic NaOH solution were poured. The yellow color was appeared by adding 2-3 drops of concentrated HCl in reaction mixture which showed the presence of coumarins.

Test for Gum and Mucilage: In 10mL of each extracts, added 1mL distilled water and 2.5mL absolute alcohol with continuous stirring. The formation of cloudy precipitates specified the availability of gum and mucilage.

Test for Terpenoids (Salkowski Test): In 5mL of each extracts, 2mL chloroform was added and then poured 3mL concentrated H_2SO_4 along the sides of test tubes deliberately to form a layer. The appearance of reddish brown color in the inter face was indicated the availability of terpenoids in extract.

Test for Alkaloids: In 2mL of each extracts, concentrated HCl (2mL) and 3-4 drops of Mayer's reagent were added. The formation of cream color showed the availability of alkaloids.

C. Quantitative Phytochemical Analysis

Total Phenolic Content Analysis (TPC): The TPC in WSJP (Wheat Seedling Juice Powder) extracts was examined by using Folin-Ciocalteu reagent by following the method of Singleton *et al.*, (1999) [20] with some alterations. The 100µL of each extracts (100µg/mL) was mixed with 900µL distilled water and then added 0.5mL of 10% Folin-Ciocalteu reagent. After adding 1.5mL of 20% sodium carbonate mixed the reaction mixture thoroughly and incubated in dark for 2 hours. When incubation period was completed make total volume to 10mL with distilled water and absorbance taken at 765nm by spectrophotometer by using gallic acid as standard. Total phenolic content was determined from gallic acid standard curve and results represented as gallic acid equivalent i.e. GAE mg/g dry weight of extract.

Total Flavonoid Content Analysis (TFC): Total flavonoid content in WSJP (Wheat Seedling Juice Powder) extract was assessed by following the procedure of Kim *et al.,* (2003) [21] with some changes. The 1mL each extracts (100µg/mL) was taken into clean test tubes and added 4mL distilled water in it. After adding 300µL $NaNO_2$, 300µL $AlCl_3$ and 2mL of 1M NaOH the reaction mixture was kept at dark incubation for 10 minutes. Then poured distilled water in test tubes to make total volume up to 10mL and absorbance read at 510nm spectrophotometrically. The results were represented as rutin equivalent *i.e.* RE mg/g dry weight of extract by analyzing through standard calibration curve of rutin.

D. Polyphenols Analysis by RP-HPLC-PDA

The RP-HPLC-PDA (Reversed-phase high-performance liquid chromatography equipped with photodiode array detector) method based analysis have been carried out to identify and quantify the various polyphenolic compounds in WSJP (Wheat Seedling Juice Powder) extract which recognized as most abundant secondary metabolites of plants and having tremendous benefits due to their anti-oxidant and oxidative stress releasing properties.

Chemicals Used: The HPLC grade solvents like methanol and water were obtained from Merck, Life Science Pvt. Ltd. Mumbai, India. The standards of polyphenols like gallic acid, caffeic acid, rutin, epicatechin, syringic acid, catechin, isoquercetin, luteolin and cinnamic acid were purchased from Sigma-Aldrich, USA.

Sample Preparation: Wheat seedling juice powder (WSJP) based extracts were formulated by sonication using methanol and water as solvents and dried on

rotary evaporator at 45°C. Then various concentrations of sample were prepared by dissolving each dry extracts in methanol (HPLC grade) and filtered across 0.22µm filter. Stock solutions of standards were prepared as 1mg/2mL i.e. 1mg of each standard dissolved in 2mL methanol : water (50:50 v/v). Various concentrations (1.0-5.0µg/mL) were prepared to form standard curve.

HPLC (High-Performance Liquid Chromatography) Instrumentation: The HPLC based polyphenolic analysis of extracts was accomplished by Waters Semi Prep HPLC system equipped with Waters 2998 Photodiode Array Detector (PDA), 1525 binary pump and temperature controller module-II column oven. Samples solutions were filtered by 0.22µm filters (Millipore, India) and analysis carried out through Waters 2707 auto sampler and Waters Empower 3 software. Separation was completed in a Luna C18-(2) 100A (250 × 4.6 mm, 5.0µm particle size) column of Phenomenex® (USA) by using methanol: water with 0.3% acetic acid (70:30, v/v, pH 3.2) as mobile phase. The isocratic flow rate was maintained as 1.0mL/min at 2200 Psi by keeping column temperature constant at 40°C. The 5µL sample was injected for analysis. The method has already been developed and followed as it is [22, 23].

Standard Curve: Stock solutions of polyphenols as 1mg/2mL were prepared by immersing 1mg of each standard in 2mL methanol: water (50:50v/v) solvents. Various concentrations ranging from 1.0µg/mL to 5.0µg/mL were taken for preparing standard curve. The curve depicted peak area *vs* concentration of standard was used for linear regression analysis which assisted to determine the analytes.

E. Statistical Analysis

Results expressed as mean ± standard deviation and best fit method applied for regression analysis. The IC_{50} value was determined by regression equation that represented 50% inhibition concentration.

III. RESULTS AND DISCUSSION

Phenolic constituents well known as secondary metabolites occurred ubiquitously in plants that protected the plants from harmful radiations and pathogen aggressiveness. The phenolic acids categorized as derivatives of benzoic acid and hydroxylcinnamic acid which familiarized for their analgesic, tranquilizer, anti-pyretic, and antibiosis characteristics [24]. Variations in the concentration of phenolic components depended on genotype, growth, harvesting and environmental conditions [25]. Many polyphenolic compounds existed as natural anti-oxidants which analyzed in lyophilized wheat seedling juice powder extract. In previous finding the free radical scavenging potential of WSJP (wheat seedling juice powder) extract was determined by DPPH assay. The extract prepared by mixed concentrations of methanol and distilled water in 80 : 20 ratio was revealed 50.72% (IC_{50} of 980.0µg/mL) radical scavenging capacity as compared to standard ascorbic acid (IC_{50} of 28.106µg/mL) [26]. Different solvents such as hexane, ethyl acetate, methanol and water were taken for wheat seedling juice powder based extract formulation. These extracts used for phytochemical analysis at preliminary basis by following various chemical tests. Anthraquinones, coumarins, alkaloids and flavonoids determined in hexane extract and flavonoid content was also analyzed in ethyl acetate extract. Methanol extract revealed the presence of glycosides, phenols, flavonoids, coumarins, alkaloids and glycosides. Carbohydrates, sugars, saponins, phenols, flavonoids, coumarins and terpenoids constituents detected in aqueous extract (Table 1).

Quantitative analysis of phenols and flavonoids was carried out by following spectrophotometric methods. Methanol extract of wheat seedling juice powder (WSJP) has highest phenolic content (56.67 ± 5.77 GAE mg/g of dry extract) i.e. followed by aqueous extract (46.7 ± 6.65 GAE mg/g of dry extract) but did not observed in case of hexane and ethyl acetate extract (Fig. 2). The flavonoid content was examined as RE mg/g of dry extract in order, Ethyl acetate extract > Hexane extract > Methanol extract > Aqueous extract. Ethyl acetate extract of WSJP carried 0.313 ± 0.058 RE mg/g flavonoid content i.e., more than hexane extract (0.103 ± 0.025 RE mg/g of dry extract). Flavonoid content in WSJP methanol extract was observed as 0.04 ± 0.027 RE mg/g and in aqueous extract as 0.007 ± 0.006 RE mg/g (Fig. 4). Total phenolic content was determined from gallic acid standard curve ($y = 0.001x - 0.000$; $R^2 = 0.998$) and flavonoid content analyzed through standard calibration curve of rutin ($y = 0.000x + 0.001$; $R^2 = 0.988$) (Fig. 1 and 3).

Table 1: Qualitative Analysis of Wheat Seedling Juice Powder (WSJP) Extracts.

Constituents	Hexane extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Carbohydrates	-	-	-	+
Proteins	-	-	+	-
Sugars	-	-	-	+
Anthraquinones	+	+	+	-
Glycosides	-	-	+	+
Saponins	-	-	+	+
Phenols	-	-	+	+
Flavonoids	+	+	+	+
Tannins	-	-	-	-
Fixed oils	-	-	-	-
Coumarins	+	+	+	+
Gum and mucilage	-	-	-	-
Terpenoids	-	-	-	+
Alkaloids	+	-	+	-

+ve sign showed presence and –ve sign showed absence of phytoconstituents.

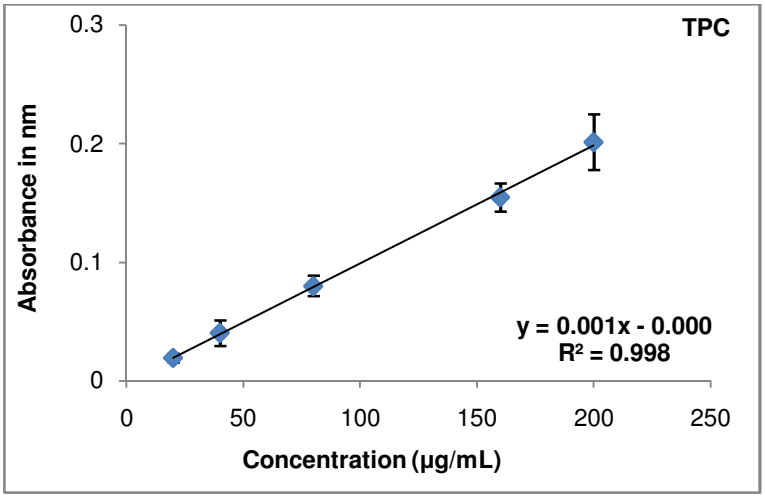

Fig. 1. Calibration curve of gallic acid for phenolic content analysis.

TPC-Total phenolic content

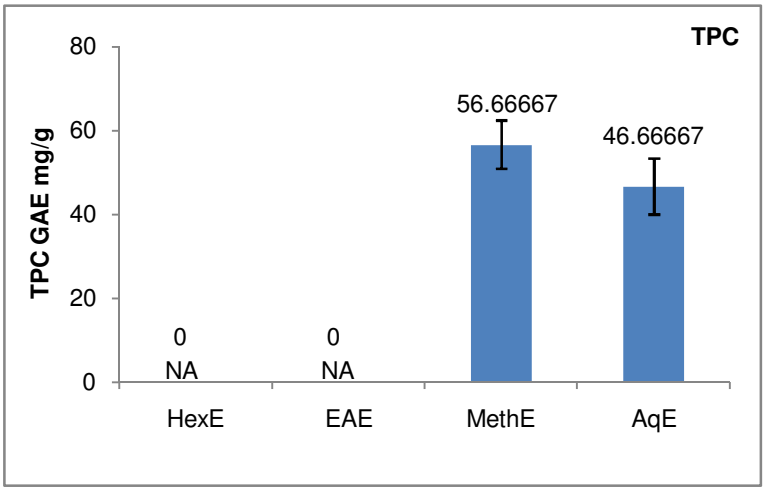

Fig. 2. Total phenolic content (TPC) as gallic acid equivalent (GAE mg/g) of dry extract in wheat seedling juice powder extracts. HexE-Hexane extract, EAE-Ethyl acetate extract, MethE-Methanol extract, AqE-Aqueous extract, NA – Not analyzed. Results as mean ± standard deviation.

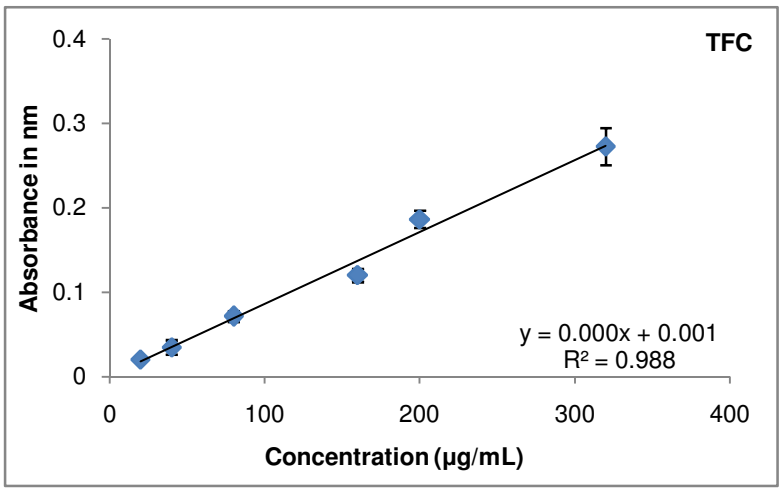

Fig. 3. Calibration curve of rutin for flavonoid content analysis.

TFC-Total flavonoid content

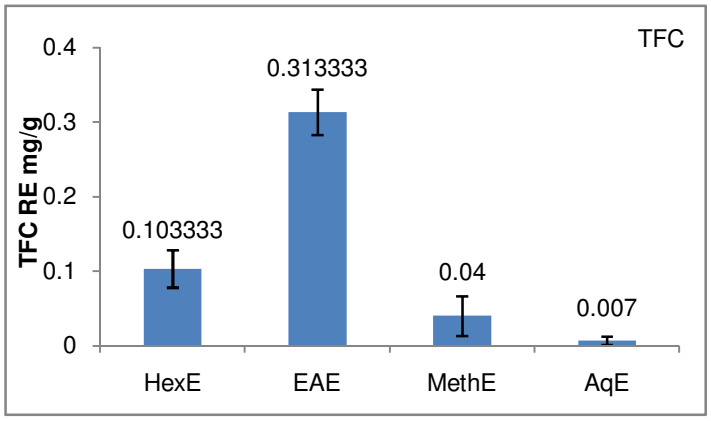

Fig. 4. Total flavonoid content (TFC) as rutin equivalent (RE mg/g) of dry extract in wheat seedling juice powder extracts. HexE-Hexane extract, EAE-Ethyl acetate extract, MethE-Methanol extract, AqE-Aqueous extract. Results as mean ± standard deviation.

On the basis of qualitative and quantitative analysis polyphenols abundantly determined in methanolic and aqueous extracts of wheat seedling juice powder so that the further analysis of some important phenolic constituents has been carried out in methanolic and aqueous extracts (80 : 20 ratio) by following RP-HPLC-PDA method. Alcoholic solvents and mixture of alcohol: distilled water observed as good extracting solvents for polyphenols [27]. The polyphenolic standards such as epicatechin, syringic acid, rutin were analyzed at 309nm and catechin, caffeic acid, gallic acid determined at 280nm but isoquercetin, cinnamic acid and luteolin at 254nm (Fig. 5). Epicatechin (2.42µg/mL) was determined as major phenolic constituent which further followed by syringic acid (1.74µg/mL) (Fig. 6). The third component was isoquercetin (1.62µg/mL) followed by caffeic acid (1.52µg/mL) but gallic acid and luteolin have been analyzed approximately equivalent i.e. 1.13 µg/mL and 1.12 µg/mL which further ensued by catechin (0.53µg/mL) >cinnamic acid (0.43µg/mL). Calibration curves for all standards and results in the form of bar diagrams are also given (Fig. 7 (I-IX) & 8).

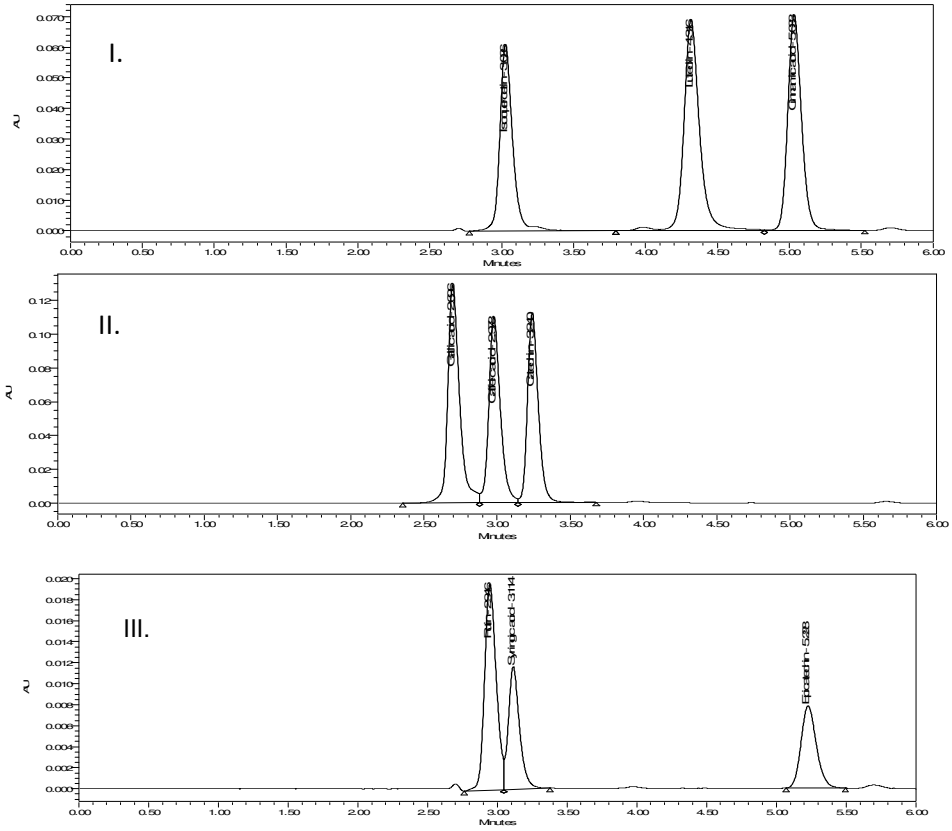

Fig. 5. (I, II & III). HPLC chromatograms of polyphenolic standards *viz.*, isoquercetin, luteolin, cinnamic acid, gallic acid, caffeic acid, catechin, rutin, syringic acid and epicatechin.

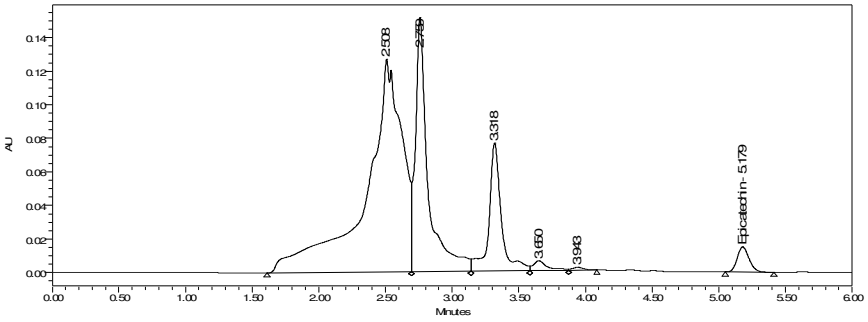

Fig. 6. RP-HPLC-PDA chromatogram of lyophilized WGJP extract having higher amount of epicatechin.

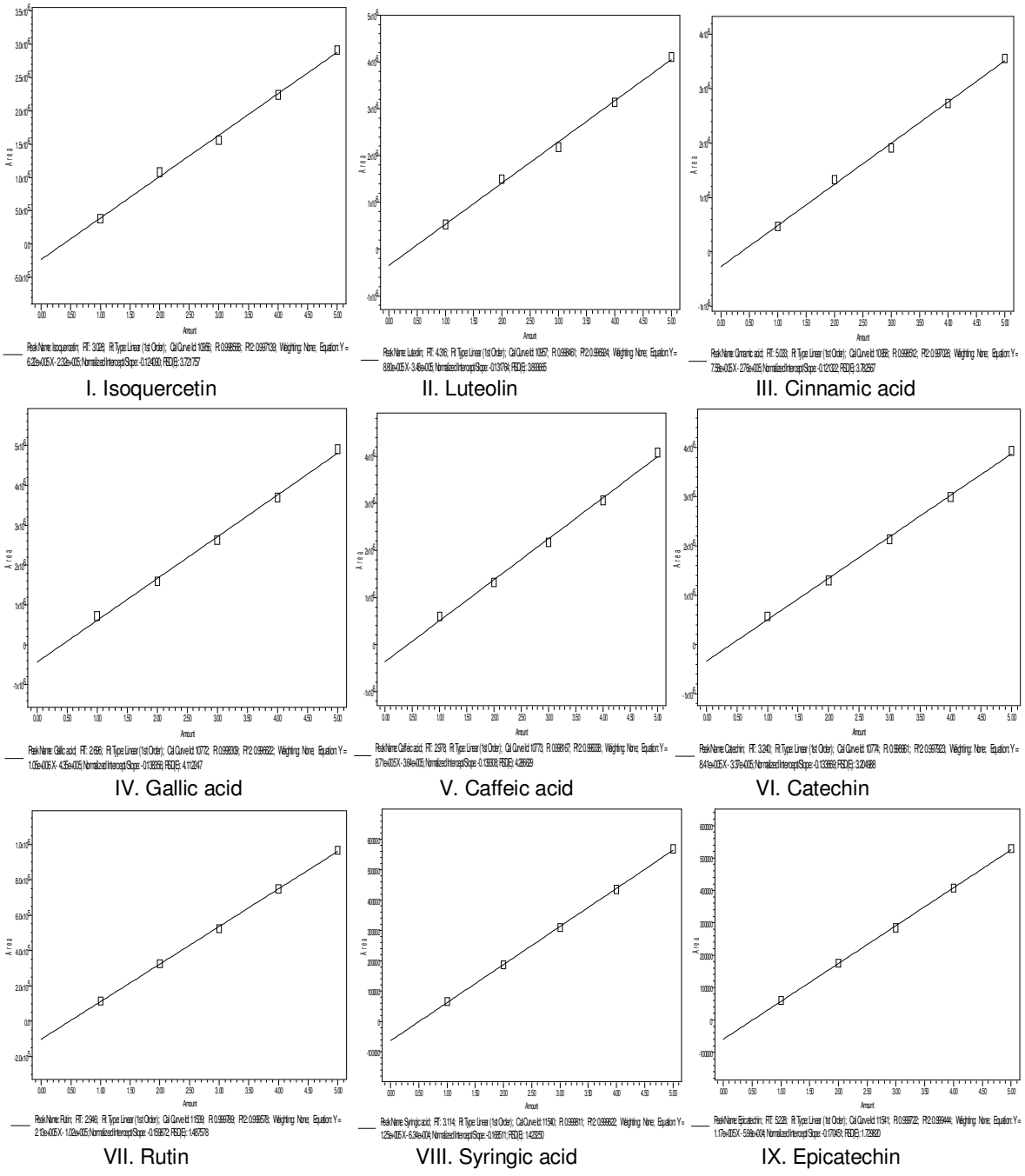

Fig. 7. (I-IX). RP-HPLC-PDA calibration curves of polyphenolic standards *viz.*, isoquercetin, luteolin, cinnamic acid, gallic acid, caffeic acid, catechin, rutin, syringic acid and epicatechin.

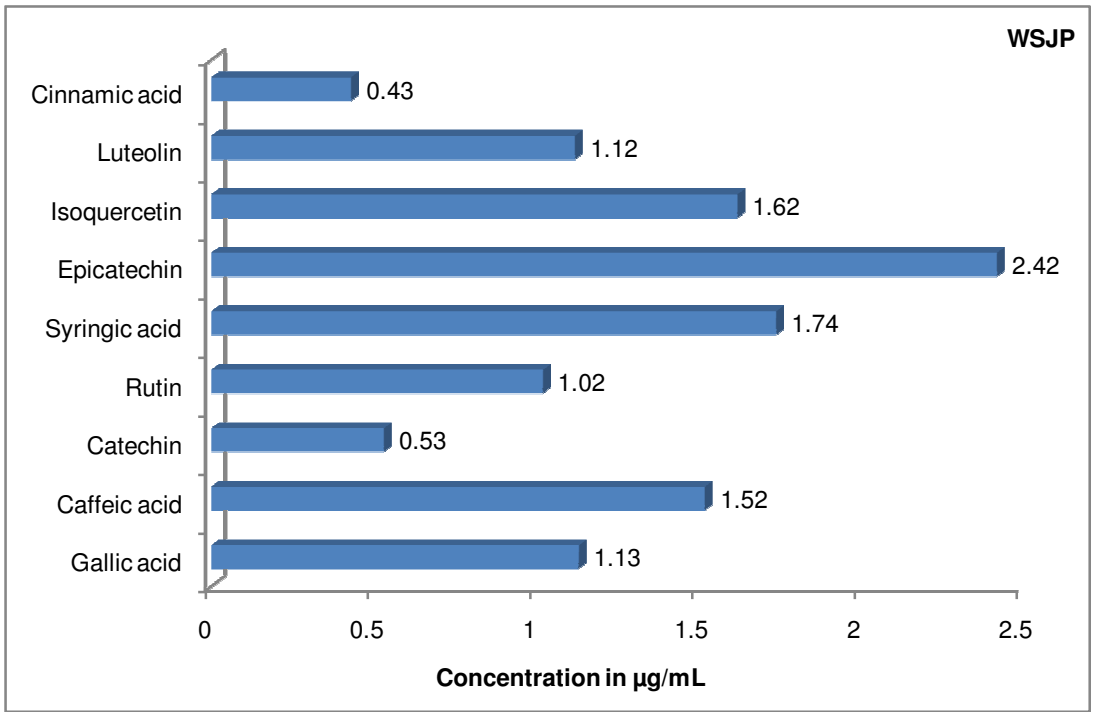

Fig. 8. Polyphenols in wheat seedling juice powder extract (WSJP-Wheat Seedling Juice Powder).

The HPLC (High-performance liquid chromatography) based method precisely separated the components on the basis of polarity of their respective extracting solvents. Epicatechin recognized as important natural therapeutic agent for lethal diseases such as diabetes and cancer. Its anti-oxidant potential facilitated to combat from cancerous diseases and capable to control the glucose level in blood of diabetes suffering peoples [28]. The second important phenolic component of WSJP, syringic acid (dimethoxybenzoic acid) recognized for cancer prevention, anti-proliferative potential, inflammation reductant, assisted to cure disorders related to cardiac, hepatic and nervous system and have neuroleptic potential by acting as transquillizer [29]. Caffeic acid is utilized in pharmaceutical and cosmetic industries due to its anti-ageing properties [30]. Rutin recognized as most important bioflavonoid with tremendous pharmacological activities aided for reducing inflammation of mucous membranes i.e. caused by chemotherapy, strengthen the blood vessels, control diabetes, cure varicose veins and healing haemorrhoids [31]. Higher quantity of phenolic constituents like ferulic acid, sinapic acid and p-coumaric acid was analyzed in wheatgrass juice powder (WJP) comparative to pulses powder (PP) [32]. Rutin and epicatechin analyzed as strong free radical scavengers [33]. The most familiar flavonoids like quercetin and apigenin possessed anti-bacterial potential [34].

IV. CONCLUSION AND FUTURE SCOPE

The methanolic and aqueous extracts of WSJP (wheat seedling juice powder) were enriched with polypenolic constituents that quantified by RP-HPLC-PDA method with high efficiency and precision. The polyphenolic components such as rutin, gallic acid, syringic acid, caffeic acid, epicatechin, catechin and luteolin have beneficial properties that aided to cure the harmful diseases by deactivating carcinogenic compounds and relieving oxidative stress. There is need to study the isolation of phytoconstituents by following other advanced chromatographic techniques to analyze volatile and non-volatile constituents.

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Conflict of Interest. No conflict of interest.

REFERENCES

[1]. Meyerowitz, S. (1999). Nutrition in Grass. *Wheatgrass Nature's Finest Medicine: The Complete Guide to Using Grass Foods & Juices to Revitalize Your Health 6th Edition edited by Book Publishing Company*, *53*.

[2]. Rana, S., Kamboj, J. K., & Gandhi, V. (2011). Living life the natural way–Wheatgrass and Health. *Functional Foods in Health and Disease*, *1*(11), 444-456.

[3]. Padalia, S., Drabu, S., Raheja, I., Gupta, A., & Dhamija, M. (2010). Multitude potential of wheatgrass juice (Green Blood): An overview. *Chronicles of young scientists*, *1*(2), 23-28.

[4]. Falcioni, G., Fedeli, D., Tiano, L., Calzuola, I., Mancinelli, L., Marsili, V., & Gianfranceschi, G. (2002). Anti-oxidant activity of wheat sprouts extract in vitro: inhibition of DNA oxidative damage. *Journal of Food Science*, *67*(8), 2918-2922.

[5]. Temple, N. J. (2000). Anti-oxidants and disease: more questions than answers. *Nutrition Research*, *20*(3), 449-459.

[6] Varjovi, M. B., Valizadeh, M. & Bandehagh, A. (2015). Primary anti-oxidant enzymes and their important role in oxidative stress in plants and mammalian. *Biological Forum–An International Journal, 7*(2), 148-154.

[7]. Zhou, K., Su, L., & Yu, L. (2004). Phytochemicals and anti-oxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry*, *52*(20), 6108-6114.
[8]. Kardas, T. A., & Durucasu, I. (2014). A new analytical method for the determination of phenolic compounds and their anti-oxidant activities in different wheat grass varieties. *Ekoloji*, *23*(90), 73-80.
[9]. Benincasa, P., Galieni, A., Manetta, A. C., Pace, R., Guiducci, M., Pisante, M. & Stagnari, F. (2015). Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species. *Journal of the Science of Food and Agriculture*, *95*(9), 1795-1803.
[10]. Puupponen, Pimia, R., Nohynek, L., Meier, C., Kahkonen, M., Heinonen, M., Hopia, A. and Oksman-Caldentey, K. M., (2001). Anti-microbial properties of phenolic compounds from berries. *Journal of Applied Microbiology*, *90*(4): 494-507.
[11]. Ghafory, H., Yousefinejad, V., Mohammadi, K., & Piri, M. (2015). Effect of Phenolic Compounds as Anti-oxidant on Cell Oxidation: A Review. *Biological Forum – An International Journal*, *7*(2), 884-887.
[12]. Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R., & Berry, E. (2002). Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. *Scandinavian Journal of Gastroenterology*, *37*(4), 444-449.
[13]. Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The American Journal of Medicine*, *91*(3), S14-S22.
[14]. Halliwell, B. A. R. R. Y., Gutteridge, J. M., & Cross, C. E. (1992). Free radicals, anti-oxidants, and human disease: where are we now? *The Journal of Laboratory and Clinical Medicine*, *119*(6), 598-620.
[15]. Zendehbad, S. H., Mehran, M. J., & Malla, S. (2014). Flavonoids and phenolic content in wheat grass plant (*Triticum aestivum*). *Asian Journal of Pharmaceutical and Clinical Research*, *7*(4), 184-187.
[16]. Ebrahimzadeh, M. A., Pourmorad, F., & Bekhradnia, A. R. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology*, *7*(18), 3188-3192.
[17]. Harborne, J. B. (1973). Phytochemical methods. *A guide to modern techniques of plant analysis*, 5-11.
[18]. Sofowora, A., (1993). Phytochemical screening of medicinal plants and traditional medicine in Africa. *Ibadan: Spectrum Books Ltd*.
[19]. Senthilkumar, P. K., & Reetha, D. (2009). Screening of anti-microbial properties of certain Indian medicinal plants. *Journal of Phytology*, *1*(3), 193-198.
[20]. Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and anti-oxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.
[21]. Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Anti-oxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, *81*(3): 321-326.
[22]. Sharma, N., Dhaliwal, H. S., & Sharma, V. (2018). Exploration of cytomorphological, genetic and phytochemical, variabilities in different species of genus *Physalis* (L.). *Thesis*.
[23]. Bano, A., Dhaliwal, H. S., & Sharma, V. (2018). Cytomorphological, phytochemical and biomolecular evaluation of germplasm of *Withania somnifera* (L.) Dunal and *Tinospora cordifolia* Miers ex Hook f. & Thoms from North-West India. *Thesis*.
[24]. Waksmundzka-Hajnos, M., Oniszczuk, A., Szewczyk, K. & Wianowska, D. (2007). Effect of sample-preparation methods on the HPLC quantitation of some phenolic acids in plant materials. *Acta Chromatographica*, *19*, 227-237.
[25]. Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and anti-oxidant activity of hard spring wheat.*Journal of Agricultural and Food Chemistry*, *54*(4), 1265-1270.
[26]. Thakur, N., Dhaliwal, H. S., & Sharma, V. (2019). Chemical Composition, Minerals and Vitamins Analysis of Lyophilized Wheatgrass Juice Powder. *International Journal on Emerging Technologies*, *10*(4), 137–144.
[27]. Mishra, R., & Ahmed, R. (2016). Phytochemical Analyais of Seeds of *Phonenix dactylifera. International Journal of Theoretical & Applied Sciences*, Special Issue-NCRTAST, *8*(1), 156-160.
[28]. Abdulkhaleq, L. A., Assi, M. H., Mohd Noor, M. H., Abdullah, R., Saad, M. Z., & Taufiq-Yap, Y. H. (2017). Therapeutic uses of epicatechin in diabetes and cancer. *Veterinary World*, *10*(8), 869-872.
[29]. Vinayagam, R. (2010). Preventive effect of Syringic acid on hepatic marker enzymes and lipid profile against Acetaminophen-induced hepatotoxicity rats. *International Journal of Pharmaceutical and Biological Archives*, *1*(4), 393-398.
[30]. Magnani, C., Isaac, V. L. B., Correa, M. A., & Salgado, H. R. N. (2014). Caffeic acid: a review of its potential use in medications and cosmetics. *Analytical Methods*, *6*(10), 3203-3210.
[31]. Moghaddasian, B., Eradatmand, A. D., & Alaghemand, A. (2013). Simultaneous determination of rutin and quercetin in different parts of *Capparis spinose. Bulletin of Environment Pharmacology and Life Sciences*, *2*(2), 35-38.
[32]. Mattila, P., Pihlava, J. M. andHellstrom, J. (2005). Contents of phenolic acids, alkyl-and alkenylresorcinols and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry*, *53*(21), 8290-8295.
[33]. Hanasaki, Y., Ogawa, S. and Fukui, S. (1994). The correlation between active oxygen scavenging and anti-oxidative effects of flavonoids. *Free Radical Biology and Medicine*, *16*(6), 845-850.
[34]. Wu, D., Kong, Y., Han, C., Chen, J., Hu, L., Jiang, H., & Shen, X. (2008). D-Alanine: D-alanine ligase as a new target for the flavonoids quercetin and apigenin. *International Journal of Antimicrobial Agents*, *32*(5), 421-426.

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