Phytochemical analysis and estimation of major bioactive compounds from *Triticum aestivum* L. grass with antimicrobial potential

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Abstract: The aim of the present study was to investigate phytochemical analysis, and qualitative and quantitative determination of major bioactive compound present in various organic extracts of *T. aestivum* L. grass. Soxhlet apparatus was used for the extraction purpose using hexane, chloroform, methanol and distilled water as a solvent system. All the extracts derived from *T. aestivum* showed qualitative presence of major phytochemicals including alkaloids, steroids and cardiac glycosides tannins, flavonoids carbohydrates. Further, HPLC analysis revealed the presence of major bioactive compounds such as rutin, chlorogenic acid, tocopherol, chlorogenic acid, and gallic acid in various organic extracts responsible for the reported maximum antimicrobial activity of *T. aestivum* grass against pathogenic bacteria including *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholerae*. These findings confirm that *T. aestivum* grass containing medicinally important bioactive compounds may have significant potential to be used in traditional medicine system for the treatment of various diseases caused by pathogenic microorganisms.

Keywords: *Triticum aestivum* L., phytochemicals, nutritive composition, HPLC, antimicrobial

INTRODUCTION

Increased prevalence of multi-drug resistant pathogens along with current appearance of microorganisms with reduced susceptibility to antibiotics has caused a significant threat for emergence of various chronic diseases resulting in the urgent need to explore effective natural remedies (Mašković *et al*., 2012). These are some reasons to divert the interest of scientists to look for an alternative in which natural products and plant metabolites are getting involved. Medicinal plants represent a rich source of secondary metabolites acting as biologically active agents with added feature of antimicrobial efficacy. It has been stated that the mechanism of the antimicrobial activity of the plant extract might be due to impaired permeability of plasma membrane, hindrance in regular cellular and metabolic processes and impairment of energy or synthesis of structural components in microbial cells (Walsh *et al.*, 2003).

Wheat (*Triticum aestivum* L.) is worldwide-cultivated food crop and the second important staple cereal food. *T. aestivum* L. is commonly known as Bread wheat and grown in India in almost all the wheat-growing zones (Stracke *et al.,* 2009). Wheat is one of the most important food crops in the world, providing 20% of humanity's dietary energy supply and serving as the main source of protein in developing nations (Braun *et al*., 2010). Wheatgrass is rich source of protein, carbohydrate, total dietary fiber, vitamin A, C, E Folic acid, niacin, riboflavin, iron, calcium, magnesium, selenium, chlorophyll and carotene each of which except carbohydrate is much more than whole cereal grain of wheat and also contains 17 amino acids, 8 of which are essential (Walters, 1992). Wheatgrass juice is a good blood building natural source and is an effective alternative of blood transfusion in terminally ill cancer and thalassemia patients (Marwaha *et al*., 2004). Moreover, wheatgrass juice has also shown the efficacy in the treatment of inflammation, and hypolipidemic properties (Kothari *et al*., 2008).

Although fewer reports proved functional potential of *T. aestivum*, current research was carried out to investigate phytochemical analysis, and qualitative and quantitative determination of majorly known biologically active compounds, present in various organic extracts of *T. aestivum* L. Grass along with their antimicrobial efficacy.

MATERIALS AND METHODS

Plant material

The seeds (10 g) of *T. aestivum* L. were collected from authentic Regional Agriculture Research Institute (RARI) for the purpose of sowing. The seeds were sown during the month of September in loamy soil (70% sand +30% clay). The pH of the soil was optimized from 6.9-7.2. The plant grass was harvested on 10th day. The harvested plant material (grass) was shade dried and grinded to powder and stored at 4°C for further analysis.

Preparation of the extracts

Maceration technique (Li *et al*., 2007) was used for the extraction. 10g powder of *T. aestivum* (grass) was suspended in 100ml of hexane followed by chloroform,

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methanol and distilled water using 250ml conical flask and kept on orbital shaker for 48 h at 37°C. After 48h, the supernatant was filtered through Whatman filter paper no.1 and evaporated to dryness at room temperature with a percentage yield of 1.65%, 0.88%, 9.8% and 12.41% (w/w), respectively. The viscous material was stored in sterile, air-tight container, and kept at 4^oC for further studies. The residue was dried and further used for successive extraction.

Preliminary phytochemical studies

Powdered material of *T. aestivum* grass (50g) was crushed and extracted with 500ml of hexane, chloroform, methanol and water using a Soxhlet apparatus. The powdered material was extracted, followed by filtration and evaporated using a rotary evaporator system. Each extract was suspended in proper solvent and to identify the phytochemical constituents present in *T. aestivum* L. grass extracts, a preliminary screening was carried by the application of various testing methods of Draggendorff's and Mayer's test, Liebermann-Bur chard test, Foam formation test, Lead acetate test, Molisch's and Felhing's test and Ferric Chloride test for determining the presence of alkaloids, terpenes, steroids, saponins, flavonoids, polysaccharides and tannins, respectively (Trease and Evans, 1983).

Quantitative HPLC analysis of phytoconstituents

Identification of components was done following the method of Hartl and Stenzel (2007) with slight modifications using HPLC analysis attached with 515 binary pumps, 2998 photodiode array detector (Waters HPLC system, Milford, USA). Various solvent systems of different solvents were used for the proper separation of the components of extract/fraction corresponding to the standard markers used in the study. The best solvent system selected as the mobile phase for the study consisted of methanol: Water in the ratio 80:20 along with 1% phosphoric acid, with a flow rate of 1.0ml/min and detection of component was achieved at 200 to 400nm at ambient temperature. The HPLC peaks of the analytes were confirmed, and quantified with known reference standards by comparing their retention times and UV spectral analysis (2998 Photodiode array detector, Waters, Milford, USA). The percentage content (w/w) of a particular phytoconstituent in a sample was calculated by putting the respective values of sample and standard in the following formula.

% Content = Sample area/Standard area × Standard weight/Sample weight × Sample volume/Standard volume × Potency/100 × 100

Microbial strains

All tested bacterial strains *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Flavobacterium species* (MTCC 2495), *Vibrio cholerae* (MTCC No. 3906), *Pseudomonas stringii* (MTCC 3365), *Salmonella typhi* (MTCC 98), *Streptococcus faecalis* (MTCC 497) and *Bacillus subtilis* (MTCC 121) were provided by the Institute of Microbial Technology (IMTECH), CG, India.

Determination of anti-pathogenic activity

Anti-pathogenic activity of extracts was performed by disc diffusion method (Ahirwal *et al*., 2011). Briefly, the extract samples were dissolved in 5% dimethyl sulphoxide (DMSO), and further diluted to obtain desired concentrations (5, 10, 20 and 50mg/ml). The bacterial strains were sub-cultured by inoculating a single colony of the bacterial inoculum in the nutrient broth (30ml) to get the viable count of 10^6 cells/ml. From this, inoculum of bacterial strains (0.1ml) was inoculated into the Molten-Muller Hinton agar medium. The discs (6mm) were impregnated with 40µl of test sample and allowed to dry for 5min. Then, the disc containing sample was placed on the seeded agar plates and allowed to stand for 1h for pre-diffusion of the extract and incubated at 37°C/24 h. Antimicrobial evaluation was performed based on the diameters of zones of inhibition around the disc and measured in mm. All experiments were done in triplicates. Streptomycin was used as a positive control, while DMSO (5%) was used as negative control.

STATISTICAL ANALYSIS

The mean values were calculated and data were analyzed using one-way analysis of variance (ANOVA), followed by Student's t-test to find out the significance at $p<0.05$.

RESULTS

Phytochemical analysis

Preliminary phytochemical studies unfolded the facts that hexane extract was found to contain alkaloids, steroids and cardiac glycosides while chloroform extract contained alkaloids, tannins, flavonoids and cardiac glycosides. Flavonoids and carbohydrates were found to present in methanolic extract while, carbohydrate was detected only in aqueous extract (table 1).

Table 1: Phytochemical analysis of different extracts of *T. aestivum* according to Trease and Evans, 1983 procedures for each type of phytochemicals.

Constituents/Test	Extracts			
	Hx	Chl	Meth	Aqu
Alkaloids	+	+	+	-
Tannins	-	+	-	-
Saponins	-	-	-	-
Steroids	+	-	-	-
Terpenoids	-	-	-	-
Flavonoids	-	+	+	-
Cardiac glycosides	+	+	-	-
Carbohydrates	-	-	+	+

+: Present; **-** : Absent; Hx: Hexane, Chl: Chloroform; Meth: Methanol; Aqu: Aqueous.

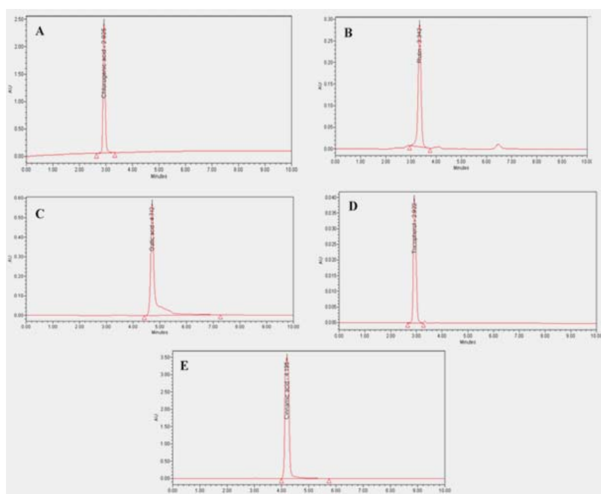

Fig. 1: HPLC chromatogram of reference standards tested (A) chlorogenic acid; (B) rutin; (C) gallic acid; (D) tocopherol; and (E) cinnamic acid

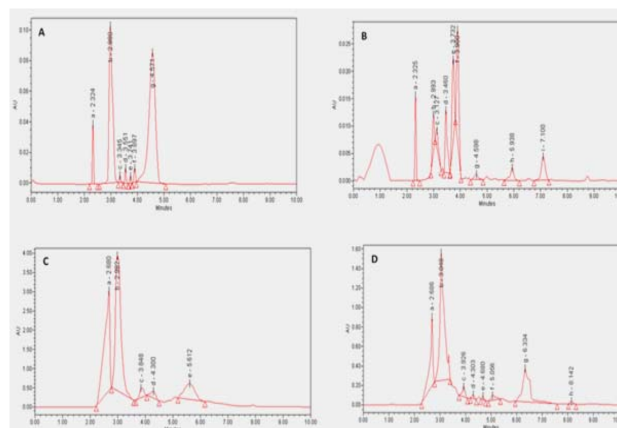

Fig. 2: HPLC chromatogram of (A) hexane; (B) chloroform; (C) methanolic; and (D) Aqueous extract of *T. aestivum*

Quantitative HPLC analysis of major bioactive components

The qualitative and quantitative estimation of rutin, chlorogenic acid, gallic acid, tocopherol, cinnamic acid and catechol as the major bioactive components in different extracts of *T. aestivum* grass was performed by using same solvent system used for samples for proper separation of the different peaks. These standard compounds were also injected separately in the column one by one using the solvent system as a mobile phase. All the standards showed well characteristic peaks at different retention times (fig. 1).

A mobile phase of methanol-water (80:20, v/v) with 1% phosphoric acid was found to be the optimum solvent system for the detection of these standards in the extract samples. A good separation was achieved for extract samples within 10 min using the above-described conditions.

The chromatogram of plant extracts (fig.2) showed different peaks at retention time corresponding to rutin (3.345), chlorogenic acid (2.982), gallic acid (4.627), tocopherol (2.993), cinnamic acid (4.115) and catechol (3.180). The presence of these phytoconstituents was further confirmed by their absorption wavelengths, which were comparable with the wavelengths of respective standards. The percentage content (w/w) of these phytoconstituents in the extracts was calculated from the obtained percentage area of reference standard vs percentage area of sample tested (table 2).

Anti-pathogenic activity

In the present study, hexane extract (200mg/ml) of *T. aestivum* L. showed the most significant activity against *V. cholerae* followed by *Staphylococcus aureus* with diameter of zone of inhibition of 8±0.5 and 7±0.5mm, respectively (table 3). On the other hand, the chloroform extract showed most promising activity against *S. typhi* with inhibition zone of 6.7±0.3 at 100mg/ml, and 7±0.5mm at 200mg/ml, respectively. Methanolic and aqueous extracts could not show any antibacterial activity.

DISCUSSION

As mentioned by several researchers, extraction is generally influenced by the sample nature, particle size, solvent type as well as extraction techniques employed. Soxhlet, heated reflux extraction and maceration are conventional procedures frequently used to recover phytochemical compounds from plant samples. The Soxhlet and heated reflux methods are normally performed at 90°C for several hours while maceration is performed over days at ambient temperature. These methods are simple, require relatively cheap apparatus and result in adequately high phenolic extraction rates (Khoddami *et al*., 2013). Castro-Vargasa *et al.* (2010) reported that the highest total phenolic content of Guava seed extract was achieved using Soxhlet extraction techniques.

Similar to our research, Sharma *et al*. (2012) used 6 different solvent systems and reported the occurrence of phenols, flavonoids, tannins, phytosterols, sennosides, carbohydrates and proteins in *Cassia pumila*. Similarly, Vadivel *et al*., (2012) also detected the presence of alkaloids, glycosides, carbohydrates, phytosterols, flavonoids, fixed oils/fats and phenolics/tannins in the leaf extracts of *Lannea coremmandelica* which have been found to exert significant antimicrobial properties.

Mašković *et al.* (2012) also reported HPLC-assisted isolation of various major phenolic bioactive compounds such as rosmarinic acid, ferulic acid, chlorogenic acid and

Table 2: HPLC analysis of *T. aestivum*.

Extracts	Retention time	Peak area	Active component	Percentage (%)
Hexane extract	i) 3.345 ii) 2.980	15083 923648	Rutin Chlorogenic acid	0.03 0.35
Chloroform extract	2.993	32509	Tocopherol	0.55
Methanol extract	2.982	49825310	Chlorogenic acid	19.17
Aqueous extract	4.680	191101	Gallic acid	0.15

Table 3: Antibacterial activity of different extracts of *T. aestivum*

Extract	Concentration (mg/ml)	Zone of Inhibition (mm)*							
		Flavobacterium sp.	E. coli	P. stringii	B. subtilis	S. typhi	S. aureus	S. faecalis	V. cholerae
Hexane	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	7.0±0.5
	100	-	-	-	-	-	-	-	7.3±0.2
	200	-	-	-	-	-	7.0±0.5	-	8.0±0.0
Chloroform	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-
	100	-	-	-	-	6.7±0.3	-	-	-
	200	-	-	-	-	7.0±0.5	-	-	-
Methanol	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-	-
Aqueous	10	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-	-

*Clearance zone including the diameter of the sterile disc (6mm).

quercetin in *Halacsya sendtneri* extracts responsible for the reported antimicrobial activity.

Current antimicrobials covering a wide array of targets are encountered as one killing the organisms and the other which merely inhibit the growth (Walsh, 2003). Antimicrobial action generally falls within one of the four mechanisms, three of which involve the inhibition or regulation of enzymes involved in cell wall biosynthesis, nucleic acid metabolism and repair, or protein synthesis, respectively. However, interaction studies based on antimicrobial targets have pre-dominantly confirmed their mode of action via inhibition of DNA replication and inhibition of cell wall integrity, and inhibition of protein synthesis (Walsh, 2003). In addition to this, the seeds of *T. aestivum* also possess antimicrobial effects against pathogenic bacteria or fungi due the presence of majorly active peptides such as Puroindolines A and Puroindolines B (Ghobad *et al.,* 2014). In earlier studies Dubreil *et al*. (1998) isolated and observed antimicrobial activity of major isoforms of puroindolines (puroindoline-a, and puroindoline-b) of *T. aestivum* seeds. Ghobad *et al.* (2014) also reported the presence of antimicrobial peptides in *T. aestivum* seeds.

Basically, the antimicrobial activity of the medicinal plants is believed to be due to the presence of secondary metabolites, which act either individually or in complex (Joshi *et al*., 2011).

These secondary metabolites include the phytochemicals, whose antimicrobial potentials have already been reported by many researchers (Yousuf *et al.,* 2012; Sharma *et al.,* 2012).

The leaves of *Hypericum perforatum* showed the presence of tannins and carbohydrates, which may be the possible components for its antimicrobial activity (Yousuf *et al*., 2012).

Maatalah *et al*. (2012) depicted the antimicrobial activity of *Anabasis articulate,* which might be due to the presence of alkaloid and saponin components. Similarly, Ahirwal *et al.* (2011) reported the antimicrobial efficacy of *Swertia chirata,* and suggested that tannins, glycosides, alkaloids and flavonoids might be responsible for its antimicrobial action.

In the present work, phenolic, flavonoids tannins and terpenoids might inhibit microbial growth by disrupting

the cell wall, binding to the adhesion complex with the cell wall. In addition, several other researchers also have shown remarkable ability of tannins to inhibit cell was protein synthesis as also confirmed previously (Maatalah *et al*., 2012).

CONCLUSION

This study showed the presence of various phytochemicals present in the various organic extracts of *T. aestivum*. In addition, determination of major bioactive components by HPLC analysis revealed the presence of rutin, chlorogenic acid, tocopherol, chlorogenic acid and gallic acid in various extracts of *T. aestivum*. The antimicrobial efficacy of *T. aestivum* against pathogenic microorganisms could be assigned to number of phytochemicals such as flavonoids, alkaloids, tannins, terpenoids and glycosides as well as major bioactive compounds present in various organic extracts of *T. aestivum*. These findings conclude that wheatgrass obtained from 10 g of seeds can be used as a dietary supplement to provide relief to the patients suffering from diseases caused by pathogenic bacteria up to certain extent.

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