Research Article

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Pharmacognostical and preliminary chemical analysis to derive quality standards of *Godhuma patra* (*Triticum aestivum* Linn. leaf)

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ABSTRACT

There are several claims that *Godhuma patra* (GP) – tender wheat – *Triticum aestivum* Linn. is a safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, anaemia, asthma and eczema. GP is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value. To sustain its valuable contribution in allaying disease in this modern era it was expected an imminent need for a well-co-ordinated research plan on herbal drug. Keeping all this in mind, pharmacognostical and analytical study including HPTLC finger printing of GP was undertaken by making use of various parameters to standardize & authenticate in accordance to international standards and quality control of *Ayurvedic* drug. Macroscopy and microscopy results have been reported here. Physico-chemical tests revealed constants for routine analysis of GP. Preliminary phytochemical analysis of aqueous extract showed presence of carbohydrate / glycoside, steroids, saponins, tannins, flavonoids, phenol and coumarins whereas ethanol extract showed presence of alkaloid and carbohydrate / glycoside. HPTLC finger print profile of ethanol extract of GP showed maximum compounds under 254 nm frequency i.e. 11 compounds, while densitometric scan showed the maximum peaks at 366 nm i.e. 14 peaks. This study carried out on GP not only established the data that maybe utilized for identification, but also established the monographic data on purity and standard of the leaf sample.

Keywords: Analytical, Herbal monographs, Pharmacopoeia, Quality standards, Standardisation.

INTRODUCTION

G*odhuma* (Wheat - *Triticum aestivum* Linn.), a cereal grass of the Poaceae family, is the world's largest edible grain cereal-grass crop since the beginning of agriculture. *Godhuma* (Wheat) is one of the non-controversial drugs and extensively consumed as *Ahaara* (Food) since ancient time till date. But the use of *Patra* (Leaf) in therapeutics has come in lime light in past several years. Although, there are numerous varieties of wheat, *Triticum aestivum* Linn. is among the most used ones. Young shoot of *T. aestivum* (*Godhuma patra* - GP) is commonly addressed as wheatgrass, belonging to family Poaceae. Wheatgrass is believed to be having manifold pharmacological diversities in additional to its nutritional value. The movement for consumption of wheatgrass began in the western world in the 1930s and was initiated by Charles F. Schnabel, known as the "Father of wheatgrass". He said ''Fifteen pounds of wheatgrass is equivalent to 350 pounds of the choicest vegetables'' [1]. Later Dr. Wigmore (1940) healed herself of cancer from the weeds she found in vacant lots in Boston. She began a study of natural healing modalities—and with the help of a friend, Dr. Earp Thomas, she found that there are 4700 varieties of grass in the world and all are good for man. Dr. Wigmore reported that the wheatgrass used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity [2]. There are several other claims that Wheatgrass is a safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, anaemia, asthma and eczema [2]. Wheatgrass is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value [3]. Moreover it is extensively available, cost-effective, can be easily grown (even at home in trays) in 8 to 10 days and fresh juice can be readily extracted everyday with minimum effort [4].

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Keeping all this in mind, pharmacognostical study, analytical study and HPTLC finger printing of GP was undertaken by making use of various parameters to standardize & authenticate in accordance to international standards and quality control of Ayurvedic drug. By standardizing the raw drugs, methods and formulations we can provide standard parameters to assess the quality, safety and efficacy of medicines [5].

MATERIALS & METHODS

Collection of sample

The authenticity of *Triticum aestivum* Linn. grains and leaves were confirmed botanically by experts and their characters were compared with various floras and standard herbarium sample available at S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi with the help of Pharmacognosist. The grains were soaked in water at room temperature for 12 hours. Afterwards the water was strained and the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. Water was sprinkled over the cotton cloth at least thrice during germination period as moisture and warm temperature are needed during the germination period. After 12 hours of germination, the germinated grains were sown in a shady place in plastic trays (30cm x 20cm), on the seventh day, the grass is harvested when it is 15 to 18 cm high [4,6].

Preservation of sample

Few leaves of GP were properly washed and preserved in a fixation solution made by the formulation of formalin-5ml, acetic acid-5ml and 70% ethyl alcohol-90ml (FAA); it was left in FAA for about 48 hours.

Preparation of powder

GP powder was obtained by shade drying the leaf for 1 day followed by oven drying at 55 ^{o} C for 6 to 8 hours. Later it was turned into fine powder with the help to mixer grinder and stored in air tight container.

Macroscopy

The fresh leaf sample and powder of GP were keenly observed under naked eyes to record the specific botanical characters.

Microscopy

The preserved specimens of GP were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.[7]

Powder microscopy

A pinch of GP powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [7].

Physico-chemical analysis

GP powder was tested for loss on drying at 105^{o}C, total ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive which were performed as per standard protocol [8].

Preliminary phytochemical analysis

Preliminary phytochemical investigation was done to detect the presence of alkaloids, steroids, carbohydrates, tannin, flavanoids, saponins, triterpenoids, coumarins and phenols in aqueous and ethanol extract [9,10].

HPTLC finger printing

One gram of powdered GP was extracted with 10 ml of ethanol by cold percolation. 5, 10 and 20 µl of the above extract was applied on a pre-coated silica gel F254 on aluminium plates to a band width of 8 mm, using CAMAG Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (5:1). The developed plates were visualized in UV 254, 366 and after derivatisation with vanillin-sulphuric acid and scanned under UV 254 and 366. R_f, colour of the spots and densitometric scan were recorded [11,12].

RESULTS

Macroscopic characters

Wheatgrass was young grass shoot where culms are simple, hollow or pithy and glabrous. Leaves were bright green in colour, flat, narrow and having parallel venation. They were 15 to 18 cm tall and 0.3 to 0.6 mm broad (Figure 1).

Microscopic characters

On the upper surface of TS of leaf, there are series of ridges, the lower surface being almost flat (Figure 3 and Figure 4). The epidermal cells covering the ridges differed in form and arrangement from those over the furrows and along the edge of the leaf. Running along the summit of each ridge there were single row of elongated thick-walled and pitted cells alternating with hairs. On the flank of the ridge, right and left of the central line, there were 3 to 5 rows of long cells interspersed with short ones and hairs (Figure 5). Parallel to these, at the base of the ridge, there were single or double lines of stomata (Figure 8). In the furrow between two ridges there were band of three to seven rows of elongated cells, whose walls are thinner. They were not distinctly parallel to each other were bulliform cells or motor cells (Figure 4).

The trichomes were unicellular, with varying length and stoutness. Some of them were blunt on the edges of older leaves where as others were short and stout, 20 to 30 µ long, with fine curved points rendering the surface scabrid appearance (Figure 4, 5 and 8). Hairs were more on the upper epidermis than the lower epidermis.

Each stoma on the leaf consisted of two guard cells and two subsidiary cells, the guard cells being narrow, with specially thickened walls around the stomatal pore and thin-walled widely dilated ends; the pore when closed appears as a narrow slit of 30 to 40 µ long (Figure 8). The ratio of the number of stomata on the upper and lower epidermis respectively was about 10:7 (Figure 6). In the transverse section, the pores of the stomata were seen to be in communication with large intracellular cavities in the mesophyll, called lacunae (Figure 3 and 5).

The parenchyma of the leaf consists chiefly of thin-walled assimilating tissue, containing lenticular chloroplasts 4.5 to 6 µ in diameter. The cells of the chlorophyll containing tissue in the central part of the leaf were much more irregular in shape and were loosely packed, 5 large intracellular spaces between them. Chloroplasts are present in the sub epidermal layers. In each cell on the outside of stereome, and between the vascular bundles, there was a single crystal or cluster of crystals of calcium oxalate (Figure 7).

Vascular bundles were somewhat nearer to the lower surface than the upper surface of the leaf (Figure 6). All vascular bundles were

collateral, with the xylem towards the upper surface of the leaf and the phloem below (Figure 4 and 6). In the xylem there were one or two vessels 20 µ in diameter with annular or spiral thickening with narrow elliptical pits. Each bundle was surrounded by an inner and outer sheath; the inner sheath was enclosed in the vascular strand, and was composed of elongated thick-walled cells; the outer sheath was more conspicuous and consisted of thin-walled cells, almost circular in transverse section. Above and below the bundles, and arranged parallel with them along the leaf were strands of supporting tissue consisting of sclerenchymatous fibers (Figure 4).

Powder was bright green in colour (Figure 2) with a characteristic non-irritant pleasant odour, more or less sweet and bit of astringent in taste. Fragment of epidermal cells in surface view was elongated and rectangular having few numbers of stomata; trichomes were simple, uniseriate, unicellular and long with pointed end and swollen bases; smaller ones were hook-shaped with broad base while longer trichomes were more in number than smaller ones; fibers thin-walled and lignified were scattered here and there, found as single or in groups; vessels were single or together in groups of 2 to 3, pitted, reticulated and annular type; pitted vessels were more in number (Figure 9).

GP was found to be free from foreign matter such as insects, moulds, animal faecal matter and other contaminants like sand, stone and extraneous materials. The loss on drying result of GP was found to be 9.87 % w/w. Total ash value was 12.24 % w/w and showed 0.4 % w/w acid insoluble ash. In GP the water and ethanol soluble extractive value was 24 and 6.91 respectively (Table 1).

The qualitative analysis carried out to determine alkaloids by using Wagner's test and Hager's tests were positive in ethanolic extractive of GP. Carbohydrates were present in water extract as well as ethanolic extract. Carbohydrates were found positive by using Molisch's test, Fehling's test and Benedict's test in water extract whereas in case of ethanolic extract, it was positive by using Fehling's test and Benedict's test. Steroids were present in water extract. Steroids were found positive in Liebermann-Buchard test and Salkowski test. The test conducted to detect saponins present in test samples became positive in water extracts. Saponins were found positive with $NaHCO_3$ on shaking with water. There was the presence of tannins in water extract which was found positive by using $FeCl_3$. Flavonoids, phenols and coumarins were found positive in water extract when analysed using Shinoda's test, $FeCl_3$ and 2N NaOH respectively. The test using tin and thionyl chloride was negative which was indicative of absence of triterpenoids in both the extractives (Table 2 and 3). The HPTLC finger print profile of alcohol extract GP has been obtained with suitable solvent system. At 254 nm and under white light the alcohol extract showed 11 spots with different R_f values. Whereas at 366 nm and after derivatisation with vanillin-sulphuric acid it has shown 10 spots out of that 8 R_f values are same. On densitometric scan of the alcohol extract, there were 12 peaks under 254 nm, 14 peaks at 366 nm, and 12 peaks under white light (Table 4 and Figure 10).

Figure 1: Vascular bundle

Figure 2: *Godhuma patra* powder

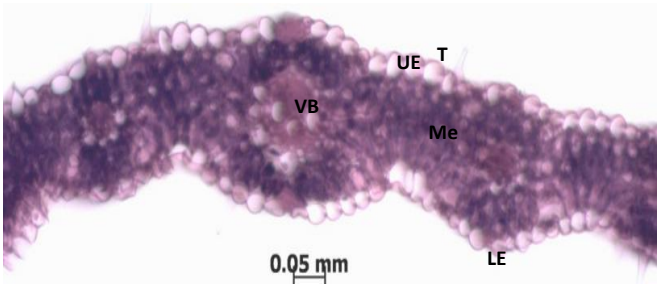


Figure 3: TS of lamina passing through midrib

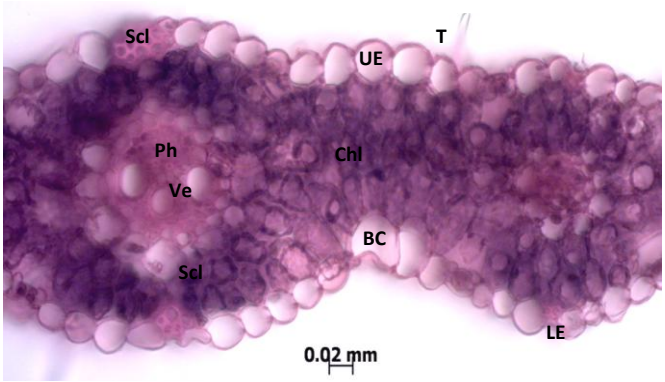


Figure 4: Portion enlarged

BC, bulliform cell; **Chl**, chlorenchyma; **LE,** lower epidermis; **Me**, mesophyll; **Ph**, phloem; **Scl**, sclerenchyma; **T**, trichome; **UE**, upper epidermis; **VB**, vascular bundle; **Ve,** vessels.

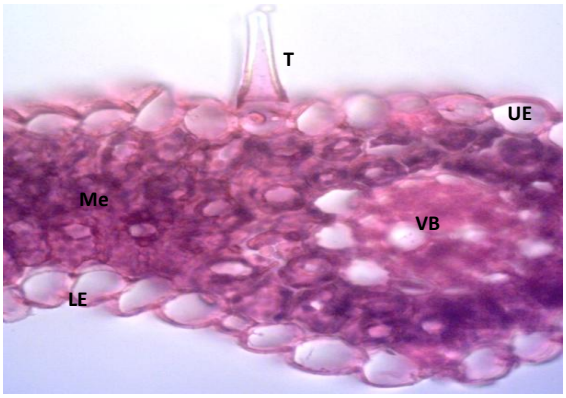


Figure 5: Midrib region showing trichome

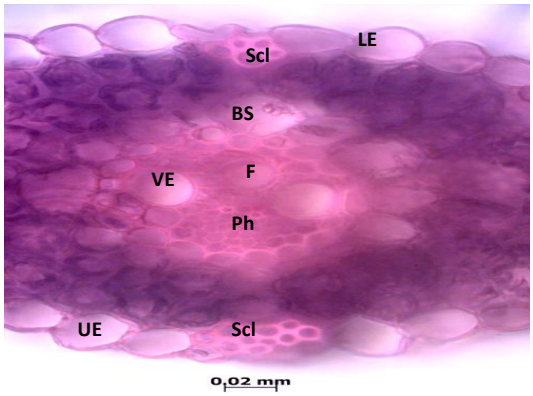


Figure 6: Vascular bundle of midrib

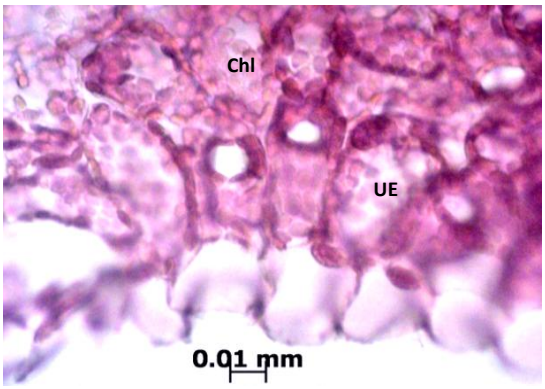


Figure7: Mesophyll

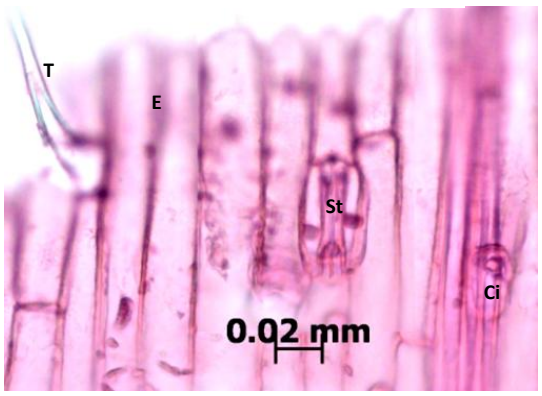


Figure 8: Epidermis in surface view

BS, bundle sheath; **Chl**, chlorenchyma; **Ci**, cicatrix; **E**, epidermis; **Me**, mesophyll; **F**, fibre; **LE**, lower epidermis; **Ph**, phloem; **Scl**, sclerenchyma; **St**, stomata; **T**, trichome; **E**, upper epidermis; **VB**, vascular bundle

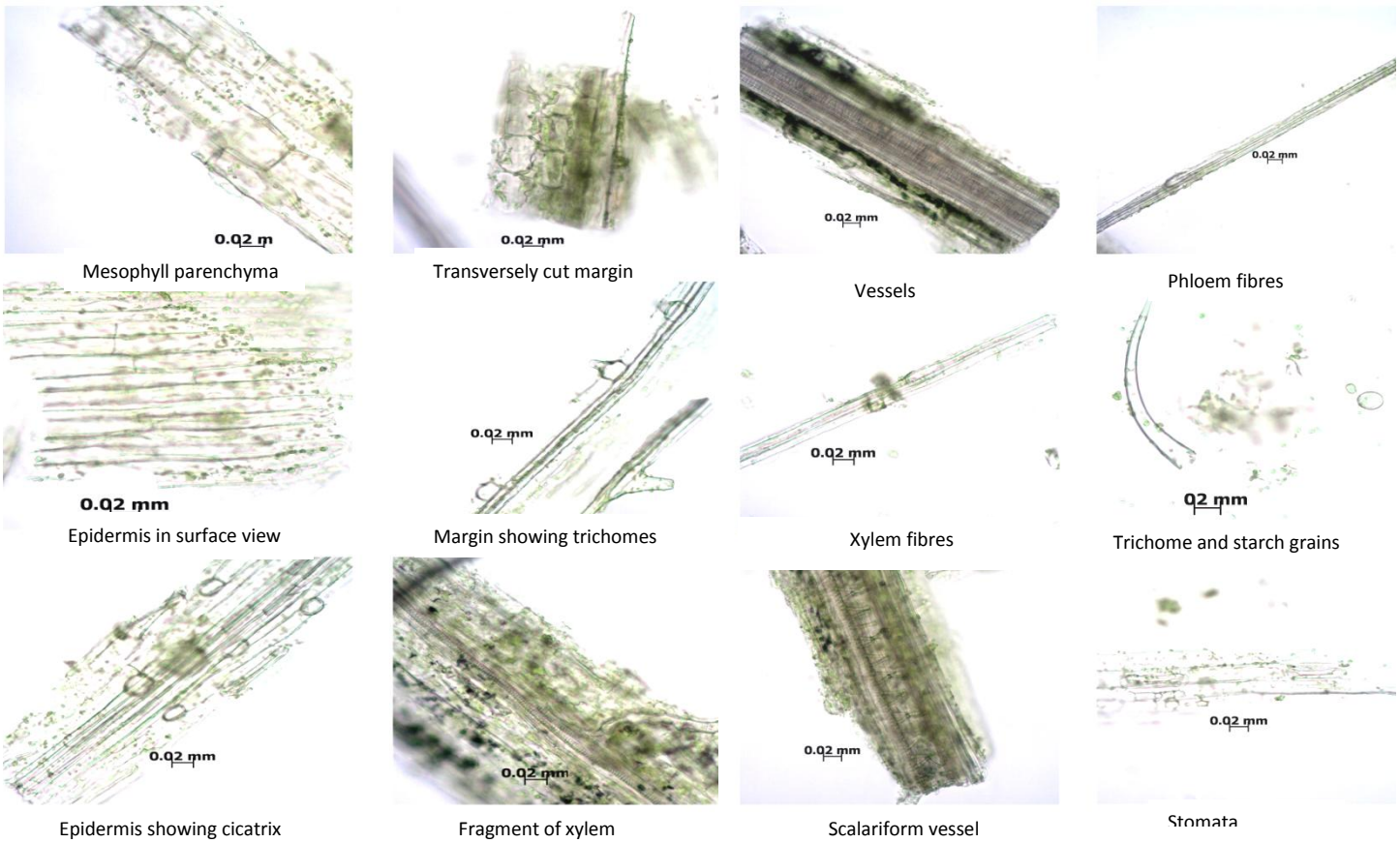
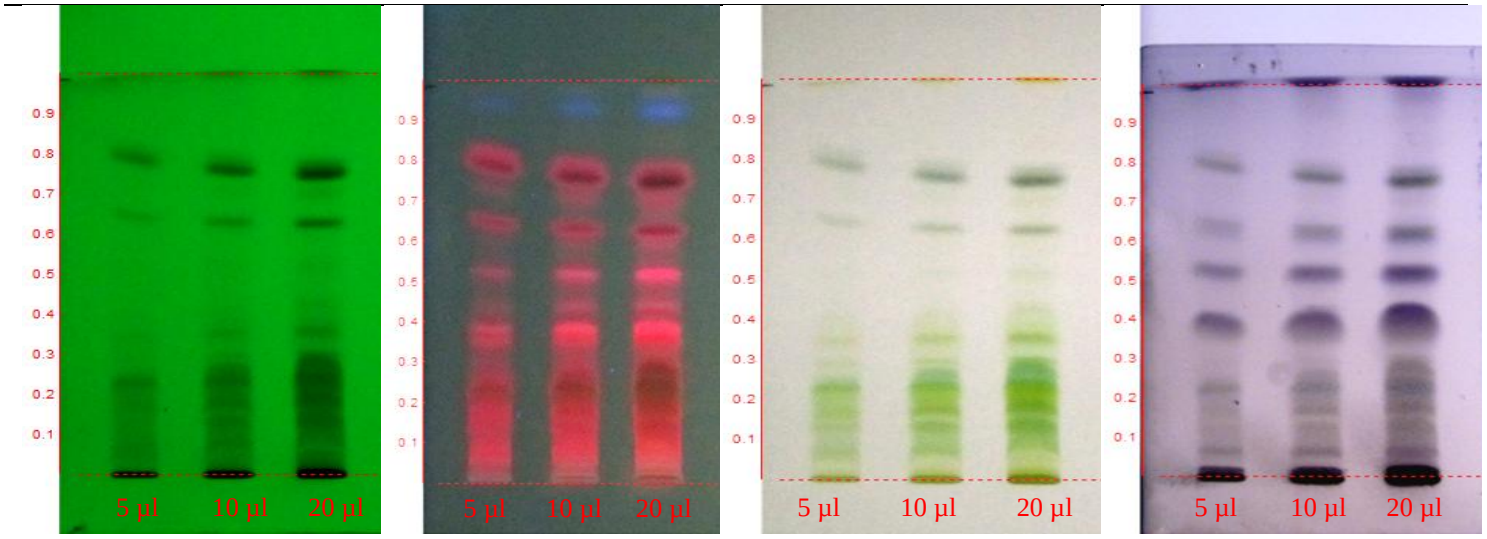


Figure 9: Microscopy of *Godhuma patra* powder

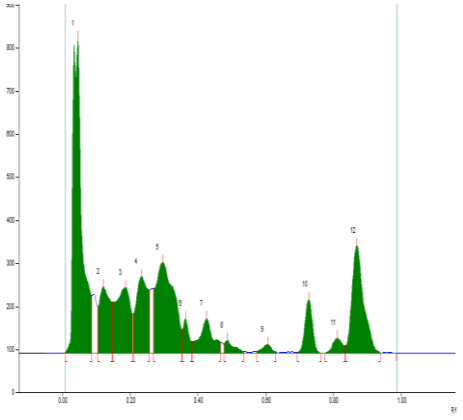


Photodocumentation at 254 nm

Photodocumentation at 366 nm

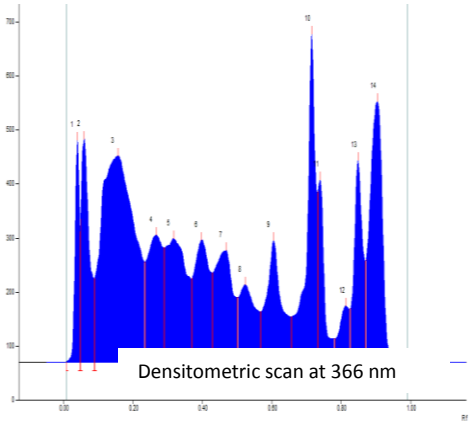
Photodocumentation under white

Photodocumentation post derivatisation



Peak	Max Position	Area %
1	0.05 Rf	29.13 %
2	0.12 Rf	7.37 %
3	0.19 Rf	10.04 %
4	0.23 Rf	9.41 %
5	0.30 Rf	16.89 %
6	0.36 Rf	2.04 %
7	0.43 Rf	4.64 %
8	0.49 Rf	1.22 %
9	0.61 Rf	0.81 %
10	0.73 Rf	4.14 %
11	0.81 Rf	1.47 %
12	0.87 Rf	12.84 %

Densitometric scan at 254 nm



Densitometric scan at 366 nm

Peak	Max Position	Area %
1	0.04 Rf	3.18 %
2	0.06 Rf	5.84 %
3	0.16 Rf	22.30 %
4	0.27 Rf	6.40 %
5	0.32 Rf	8.31 %
6	0.40 Rf	6.02 %
7	0.47 Rf	6.55 %
8	0.52 Rf	4.14 %
9	0.61 Rf	6.09 %
10	0.72 Rf	9.45 %
11	0.74 Rf	3.76 %
12	0.81 Rf	1.87 %
13	0.85 Rf	5.62 %
14	0.90 Rf	10.47 %

Solvent system: Toluene: Ethyl acetate (5:1)

Figure 10: HPTLC fingerprint of ethanol extract of wheatgrass

Table 1: Physico – chemical parameters of Godhuma Patra

Parameter	**Result n = 3** (% w/w)
Loss on drying at 105°C	9.87
Total ash	12.24
Acid insoluble ash	0.4
Water soluble extractive	24
Alcohol soluble extractive	6.91

Table 2: Observation on phytochemical tests of *Godhuma patra*

Tests	Color if positive	Water	Ethanol
Alkaloid			
Dragendroff's test	Orange red precipitate	Yellow precipitate	Green
Wager's test	Reddish brown precipitate	Brownish yellow precipitate	Reddish brown
Mayer's test	Dull white precipitate	Yellow solution	Dark green
Hager's test	Yellow precipitate	Yellow solution	Yellow precipitate
Carbohydrate			
Molisch's test	Violet ring	Dark violet colored ring	Blue
Fehling's test	Brick red precipitate	Brick red precipitate	Brick red precipitate
Benedict's test	Red precipitate	Red precipitate	Red precipitate
Anthrone- sulphuric acid test	Dark green	-	-
Steroids			
Liebermann-Buchard test	Bluish green	Bluish green	yellow
Salkowski test	Bluish red to cherry red	Bluish red to cherry red	Orange
Saponins			
With $NaHCO_3$	Stable froth	Stable froth	No froth
On shaking with water	Frothing	Frothing	
Tannins			
With $FeCl_3$	Dark blue or green color	Green color	Dark Yellow
Flavonoids			
Shinoda's test	Red to pink	Light Pink	Light yellow
Phenol			
$FeCl_3$	Blue to blue black, green	Dark green	Yellow
Coumarins			
2N NaOH	Dark yellow	Yellow color	Orange
Triterpenoids			
Liebermann-Buchard test	Pink	Light green	Light green
Tin and thionyl chloride test	Pink	Light green	Green

Table 3: Inference of phytochemical tests of extracts of *Godhuma patra*

Test	Water	Ethanol
Alkaloid	-	+
Carbohydrate / glycoside	+	+
Steroids	+	-
Saponins	+	-
Tannins	+	-
Flavonoids	+	-
Phenol	+	-
Coumarins	+	-
Triterpenoids	-	-

Table 4: R_f values of alcohol extract of *Godhuma patra*

At 254 nm	At 366 nm	Under white light	Post derivatisation
0.06 D green	0.06 F pink	0.06 Green	0.06 Purple
0.12 D green	0.12 F pink	0.12 Green	0.12 Brown
0.17 D green	0.17 F pink	0.17 Green	0.17 Brown
0.23 D green	0.23 F pink	0.23 Green	0.23 Green
0.26 D green	0.26 F pink	0.26 Green	0.26 Brown
0.30 L green	-	0.30 L green	0.39 Violet
0.35 L green	0.35 F pink	0.35 L green	-
0.52 L green	0.52 F pink	0.52 L green	0.51 Violet
0.62 D green	0.62 F pink	0.62 L green	0.62 Violet
0.76 D green	0.76 F pink	0.76 D green	0.76 Green
0.79 L green	-	0.79 Green	0.79 L green
-	0.93 F pink	-	-

Solvent system: Toluene: Ethyl acetate (5:1)

DISCUSSION

The macroscopic features recorded can be used for preliminary identification of GP. Features like unicellular trichomes, single crystal or cluster of crystals of calcium oxalate around vascular bundle, collateral vascular bundles, strands of supporting tissue consisting of sclerenchymatous fibers will be helpful in identification of GP under microscope. Bright green pleasant smelling astringent tasting powder showed epidermal cells with elongated and rectangular having few numbers of stomata, uniseriate trichomes with pointed end and swollen bases, thin-walled lignified single or grouped fibres, reticulated – annular – pitted vessels. In many of studies reported earlier, the macro-microscopic studies have been proved to be effective in establishing the authenticity and detection of adulterants/substitutes for herbal raw drugs [13,14]. The loss on drying indicates the moisture and volatile matter content in sample; result of GP was found to be 9.87 % w/w. The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts. Total ash of the drug is inclusive of physiological and non-physiological salts. physiological ash derived from the plant tissues. While the non-physiological ash consists of residue of the extraneous matter such as sand, soil, etc adhering to herb itself. In GP total ash value was 12.24 % w/w; acid insoluble ash gives a percentage of materials mainly silica which remains insoluble in acid, GP showed 0.4 % w/w acid insoluble ash. The extract obtained by percolating coarse powder is indicative of approximate quantity of their chemical constituents. Taking into consideration the diversity in chemical nature and the properties of contents of drug, various solvents were used for the determination extractives. The solvent used for the extraction is in a position to dissolve appreciable quantities of substances desired. In GP the water and ethanol soluble extractive value was 24 and 6.91 respectively. The extracts obtained by exhausting crude drug are indicative of approximate measure of their chemical constituents. Taking into consideration the diversity in chemical nature and the properties of contents of drug, various solvents are used for the determination extractives. The solvent used for the extraction is in a position to dissolve appreciable quantities of substances desired. These preliminary analyses of chemical composition is one of the best methods to analyse quality of herbs [15]. The tests showed presence of carbohydrate, steroids, saponins, tannins, flavonoids, phenols and coumarins in aqueous extract of GP and presence of alkaloids and carbohydrates in ethanolic extract of GP. The HPTLC finger print profile of alcohol extract GP was been obtained with suitable solvent system. At 254 nm and under white light the alcohol extract of GP showed 11 spots with same R_f values. Whereas at 366 nm and after derivatisation with vanillin-sulphuric acid it showed 10 spots out of that 8 R_f values were same. On densitometric scan the alcohol extract of GP under 254 nm showed 12 peaks, at 366 nm 14 peaks, under white light it showed 12 peaks. HPTLC finger print profile of alcohol extract GP showed maximum compounds under 254 nm. On densitometric scan of the GP alcohol extract, showed the maximum peaks at 366 nm. HPTLC fingerprinting is an effective technique of screening herbal raw drugs for authenticity and quality [16,17].

CONCLUSION

Adulteration is a major problem while assessing the identity and quality of herbal drugs. Pharmacognostical as well as analytical study of *Godhuma Patra* (*Triticum aestivum* Linn. Leaf) were the primary steps to establish its quality control and authentication as per WHO guidelines.

Macro-microscopy and physico-chemical constants for wheat grass has been documented. Preliminary phytochemical analysis of GP aqueous extract showed presence of carbohydrate / glycoside, steroids, saponins, tannins, flavonoids, phenol and coumarins whereas GP ethanol extract showed presence of alkaloid and carbohydrate / glycoside. HPTLC finger print profile of alcohol extract GP showed maximum compounds under 254 nm frequency i.e. 11 compounds. On densitometric scan of the GP alcohol extract, showed the maximum peaks at 366 nm i.e. 14 peaks. This study carried out on GP not only established the data that maybe utilized for identification, but also established the purity and standard of the leaf sample. Monographic data by pharmacognostical and analytical study on this herbal medicine may be used as reference standard in future studies.

CONFLICTS OF INTEREST

No conflicts of interest.

SOURCE OF FUNDING

Nil.

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