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QUALITY CONTROL STANDARDIZATION AND ANTI-INFLAMMATORY ACTIVITY OF AYURVEDA FORMULATION YASHTIMADHU CHURNA

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ABSTRACT

Yashtimadhu Churna is one of the traditional formulations used as antacid, anti-inflammatory, anticancer, anti-oxidant, analgesic, anti-stress, demulcent, antibacterial and anti-asthmatic agents. The formulation mainly contains *Yastimadhu (Glycyrrhiza glabra)* root extract. Present study described preliminary qualitative phytochemicals analysis and chromatographic fingerprinting of *Yashtimadhu Churna* using HPLC and HPTLC analysis. Study also presented anti-inflammatory potential of formulation using protein denaturation method. This study helps to establish standardization parameters of *Yashtimadhu Churna* and also confirmed anti-inflammatory potential of formulation.

Keywords : *Yashtimadhu Churna, Glycyrrhiza glabra, Standardization, Protein Denaturation.*

INTRODUCTION

Glycyrrhiza glabra (G. glabra) Linn is one of the valuable medicinal herb belongs from family *Leguminosae* and widely used in various systems of medicines world widely (Arundatta, 2009; Tatav *et al.*, 2011). The plant mainly found in China, India, United State of America and Russia, etc. (Lakshmi & Geetha 2011). The plant roots mainly contain starch, mucilage, gum, calcium, magnesium salts and saponin named glycyrrhizin (Kumar & Dora 2012).

Yastimadhu churna is traditional formulation contains root extract of *Yastimadhu (Mulethi)* used to heal gastric ulcers, it helps in gastro-esophageal reflux disorder, helps to treat throat irritation, stimulate liver function and acts as a mental rejuvenator (Lakshmi & Geetha, 2011). Therapeutically it can be used for treating cough, congestion, expel out phlegm from the lungs, treat viral and bacterial infections. It acts as a aphrodisiac agent thus treat infertility especially in male. It offers health benefits in skin problems like psoriasis, eczema, skin rashes and dry skin (Lakshmi & Geetha, 2011; Kumar & Dora, 2012).

The quality control standardization is one of the important aspects related to the Ayurveda and herbal formulation. It is very essential to establish quality parameters of Ayurveda formulations to enhance their global acceptance for therapeutic purposes. Considering these all aspect this study presented quality control standardization of *Yashtimadhu Churna*.

MATERIAL AND METHODS

The drug sample was purchased from the local market, other reagents and solvents used in study were of analytical grade, Acetonitril & other solvents for chromatographic analysis used were of HPLC grade.

Preliminary qualitative phytochemicals analysis

Formulation subjected to qualitative chemical analysis to confirm presence of phytochemicals like; phenolic compounds, flavonoids, alkaloids, saponins and tannins, etc. (Khandelwal, 2007).

Determination of total phenolic and flavonoid contents

Total phenolic content in formulation was determined by colorimetric assay (Folin Ciocalteu's method). The calibration curve of standard gallic acid was used to calculate total phenolic contents in drug sample (Lin & Tang, 2007; Alhakmanu *et al.*, 2013). The total phenolic content in formulation was expressed as Gallic acid equivalent (GAE) (mg/g of dry mass).

The total flavonoid content in formulation was determined by colorimetric method using Quercetin as standard (Ahmad *et al.*, 2014). Flavonoid content in formulation was reported as Quercetin equivalent ($\mu\text{g/gm}$ of dry mass).

HPLC Analysis of Formulation

Sample preparation

Sample (0.3 g.) was dissolved in methanol and shake for 30 minutes. Supernatant was decanted after centrifuging for 5 min. The residue mixed in methanol and decanted after shaking for 30 minutes two times subsequently. The supernatants were added after evaporating solution then filtered through a syringe filter and subjected to HPLC analysis.

Standard curve preparation

Standard solution was prepared with known amount of β -glycyrrhetic acid and serial dilution method was used to prepare different concentrations of standard solution i.e.; 100, 200, 300, 400 & 500 μ g/ml. Blank determinations also performed using methanol as solvent.

Chromatographic conditions

Shimadzu HPLC system was used containing sample loop of 20 μ l capacity with C-18 reversed phase column, LC-10AD pump and Photodiode-array detector. Acetonitril / phosphoric acid (2.5/0.5) mixture was used as mobile phase with as flow rate of 0.5 ml/min. The wavelength 230 nm was selected as detection wavelength (Shi *et al.*, 2012).

HPTLC Fingerprinting of Formulation

CAMAG HPTLC system with a Linomat sample applicator was used for HPTLC fingerprinting analysis of formulation. Ethyl acetate: Methanol (8:2) was selected as mobile phase after many trial & error experiments. The HPTLC analysis was carried out at room temperature and 5 μ L sample solution was spotted on the pre-coated silica gel plates maintaining bands width of 6 mm. The CAMAG twin trough plate development chamber was used for development of plates. The plates were air dried and scanned after completion of development process (Mauji *et al.*, 2011).

Anti-inflammatory activity of *Yashtimadhu Churna* (Protein denaturation assay)

Anti-inflammatory activity of *Yashtimadhu Churna* was evaluated using protein denaturation assay (Mizushima, 1964). Inhibition of protein denaturation by *Yashtimadhu Churna* was considered as anti-inflammatory potential of formulation. The reaction mixture consisted of 1% bovine albumin, phosphate buffered saline (pH 6.4) and formulation. The reaction mixture incubated in a water bath at 37°C for 15 min then heated at 70°C for 5 min. The turbidity of solution was measured after cooling at 660 nm using a UV/VIS spectrometer. Phosphate buffer solution was employed as control. The percentage inhibition of protein denaturation was measured using following formula:

$$\% \text{ inhibition of protein denaturation} = 100 \times (1 - A2/A1)$$

Where A1 = absorption of the control, A2 = absorption of the test sample.

RESULTS AND DISCUSSION

Preliminary qualitative phytochemicals analysis

The finding of phytochemical screening confirmed presence of carbohydrates, alkaloids, flavonoids, saponins, tannins and steroids, etc. The results of the phytochemicals analysis presented in Table 1.

Table 1: Phytochemical composition of *Yashtimadhu Churna*

S. No.	Chemical Constituent	Present/Absent
1	Carbohydrates	Present
2	Saponins	Present
3	Alkaloids	Present
4	Phytosterols	Present
5	Flavonoids	Present
6	Tannins	Present

Total phenolic and flavonoid contents

The total phenolic and flavonoids contents in formulation were represented as mean \pm S.D of triplicates. The total phenolic contents was found to be 7.55 ± 0.03 mg/gm of Gallic Acid Equivalent (GAE) and total flavonoids contents was found to be 2.33 ± 0.02 μ g/gm of Quercetin Equivalents (QE).

HPLC Analysis of Formulation

HPLC analysis observed showed chromatogram of standard (β -glycyrrhetic acid) and this peak was also observed in the HPLC graph of formulation. The HPLC chromatograms of standard and formulation depicted in Figure 1, where Figure 1 A represents HPLC chromatogram of pure standard β -glycyrrhetic acid while Figure 1 B represents HPLC chromatograms of formulation *Yashtimadhu Churna*.

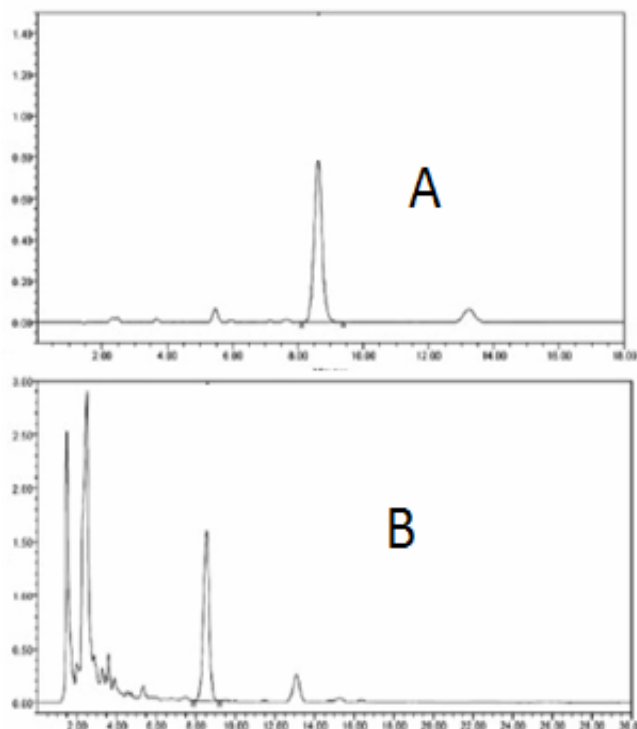


Fig. 1: HPLC chromatograms of standard β -glycyrrhetic acid (A) and formulation *Yashtimadhu Churna* (B).

HPTLC Fingerprint Profile of *Yashtimadhu Churna*

HPTLC fingerprinting of *Yashtimadhu Churna* was carried out Ethyl acetate: Methanol solvent system. Various peaks at different R_f values were observed in HPTLC chromatograms as shown in Figures 2 and 3. The peaks in HPTLC graph represent various phyto-constituents present in Ayurveda formulation.

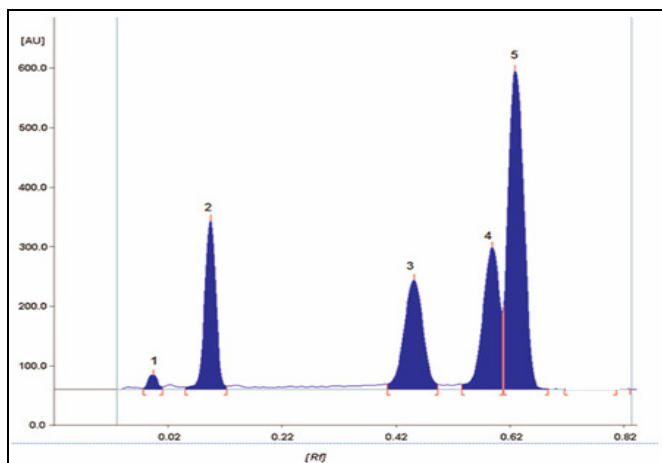


Fig. 2: Densitometric chromatogram of formulation.

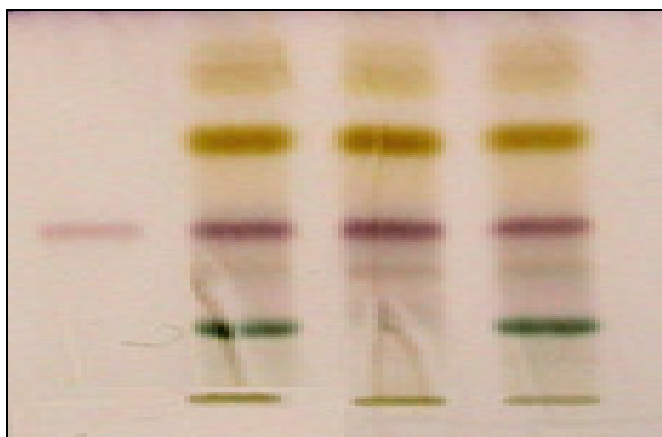


Fig. 3 : HPTLC Chromatogram of formulation

Anti-inflammatory activity of *Yashtimadhu Churna* (Protein denaturation assay)

The formulation inhibits protein denaturation in a concentration dependent manner and inhibitory effect of formulation varied depending upon concentrations of formulation. The various concentration (25, 50, 100 $\mu\text{g/mL}$) of *Yashtimadhu Churna* were used to measure inhibition of protein denaturation as anti-inflammatory potential of formulation. The maximum inhibition was observed at higher concentration as depicted in Figure 4.

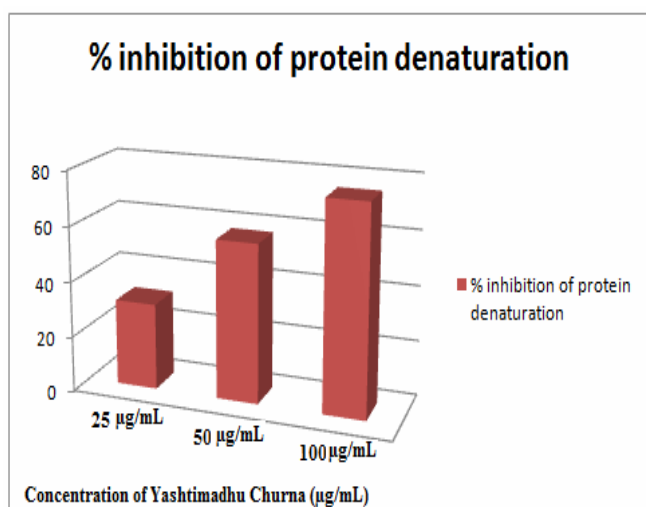


Fig. 4: % Inhibition of Protein Denaturation by Various Concentration of *Yashtimadhu Churna*.

The result of phytochemical analysis showed presence of alkaloids, flavonoids, saponins, tannins and steroids, etc. The presence of such compounds may be attributed to the therapeutic properties of formulation like antibacterial, anti-inflammatory and anticancer activities. The total phenolic and flavonoid contents also found in significant amount which enhances antioxidant capacity of formulation.

The results of HPLC and HPLTC analysis confirm presence of standard β -glycyrrhetic acid in formulation. The various peaks at different R_f values in chromatographic analysis indicated presence of active constituents in formulation. These fingerprinting images of formulation can be referred as standard reference for quality establishment of Ayurveda formulation *Yashtimadhu Churna*.

Denaturation of protein molecules is one of the reported processes related to the inflammatory condition. Inhibition of protein denaturation can be considered as anti-inflammatory response of any drug like agents (Mizushima, 1964). The present study observed dose dependent inhibition of protein denaturation by Ayurveda formulation *Yashtimadhu Churna* and this inhibition may be due to the anti-inflammatory property of *Yashtimadhu Churna*. Present of chemical constituents such as alkaloids, flavonoids, tannins and steroids, etc. can be attributed to the anti-inflammatory response of *Yashtimadhu Churna*.

CONCLUSION

Herbal drugs or Ayurveda formulations used extensively for their therapeutic properties and global acceptance of such medicine also increasing day by day. Therefore it is very essential to establish quality control parameters of natural drugs. Considering this aspect present study was planned to establish standardization parameters of Ayurveda formulation *Yashtimadhu Churna*. This study reported phytochemicals constituents present in formulation, chromatographic fingerprinting and anti-inflammatory potential of *Yashtimadhu Churna*. The parameters established in present study may be used as standardization tools for ensuring quality of Ayurveda formulation *Yashtimadhu Churna*. The study also confirmed that this compound can be used as potent anti-inflammatory agent without any severe side effects.

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