



E-ISSN: 2278-4136
 P-ISSN: 2349-8234
 JPP 2016; 5(4): 136-138
 Received: 20-05-2016
 Accepted: 22-06-2016

Gurinder Singh
 Research Scholar, Department of
 Human Genetics, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Deepak Kumar
 Research Scholar, Department of
 Pharmaceutical Sciences and
 Drug Research, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Dr. Suresh Kumar
 Assistant Professor, Department
 of Pharmaceutical Sciences and
 Drug Research, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Dr. Promila Mehta
 Professor, Department of
 Human Genetics, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Dr. Gijinder Kaur
 Assistant Professor, Department
 of Human Genetics, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Correspondence
Gurinder Singh
 Research Scholar, Department of
 Human Genetics, Punjabi
 University, Patiala – 147 002,
 Punjab, India.

Estimation of total phenols and flavonoids in Jwarnashak Panch Kashya and its antioxidant activity

**Gurinder Singh, Deepak Kumar, Dr. Suresh Kumar, Dr. Promila Mehta
 and Dr. Gijinder Kaur**

Abstract

Jwarnashak Panch Kashya is the polyherbal formulation contained five herbal drugs (*Tinospora cordifolia*, *Swertia chirata*, *Fumaria parviflora*, *Zingiber officinale* and *Cyperus rotundus*) in equal proportion has been traditionally used as antipyretic. The present research work was designed to estimate total phenols, flavonoids and antioxidant activity in selected polyherbal formulation. Properly identified powdered polyherbal formulation was extracted in Soxhlet apparatus with methanol. Ethyl acetate fraction of methanol extract was prepared using standardized procedure. Quantification of phenols and flavonoids was done on the basis of standard curve of gallic acid and quercetin, respectively, using standardized procedures. *In vitro* antioxidant activity was evaluated using DPPH assay in comparison to standard drug rutin. The total phenols and flavonoids content in methanol extract of polyherbal formulation were found to be 9.01 and 5.13% w/w, respectively, whereas total phenols and flavonoids content in ethyl acetate fraction of polyherbal formulation were found to be 17.99 and 9.11% w/w respectively. Maximum antioxidant activity was observed in methanol extract and ethyl acetate fraction of polyherbal formulation at the concentration of 20 and 5 µg/ml respectively. Therefore, it can be concluded that antioxidant activity of polyherbal formulation may be due to presence of polyphenols.

Keywords: Antioxidant activity, DPPH, Flavonoids, Phenols

1. Introduction

Combination of herbal drugs is one of the highlighted concept of Ayurvedic system of medicines to achieve maximum therapeutic response. Recently, a large number of pharmaceutical formulations like Chyawanprash, Dashmoolarista, Medhya Rasayana and Mahanarayan Taila are widely used in Ayurvedic system of medicines. The active phytoconstituents of single plant are insufficient for desirable therapeutic response. Therefore, the concept of polyherbal formulation has been more rational to give a better therapeutic response in comparison to single plant and reduce the risk of toxicity^[1].

Traditionally, Jwarnashak Panch Kashya is used in the treatment of fever. Jwarnashak Panch Kashya contains *Tinospora cordifolia* aerial parts (Guduci; Menispermaceae), *Swertia chirata* aerial parts (Chirata; Gentianaceae), *Fumaria parviflora* whole plant (Papra; Fumariaceae), *Zingiber officinale* rhizomes (Ginger; Zingiberaceae) and *Cyperus rotundus* rhizomes (Nagarmotha; Cyperaceae)^[2].

A survey of literature revealed that most of phytochemical and pharmacological research work has been carried out on selected plants in polyherbal formulation. But no phytochemical work has ever been carried out on Jwarnashak Panch Kashya. Therefore, it was planned to estimate total phenols, flavonoids content and antioxidant activity in methanol extract and its ethyl acetate fraction of polyherbal formulation.

2. Materials and methods

2.1 Plant materials

The plants of Jwarnashak Panch Kashya were procured from Himalaya Herb Stores, Madhav Nagar, Saharanpur, Uttar Pradesh, India in December, 2015. The plants were identified by Dr. Avneet Pal Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala, India (Reference No. SPL-111/Bot, dated 25-12-2015).

2.2 Preparation of extract and fraction

The methanol extract and ethyl acetate fraction of polyherbal formulation were prepared as per standard procedure using LR grade solvents of E Merck, New Delhi, India^[3]. The solvents from extract and fraction were recovered under reduced pressure using rotary vacuum evaporator (Buchi, Switzerland).

2.3 Estimation of total phenols, flavonoids content and *in vitro* antioxidant activity

The total phenols and flavonoids content were estimated using Folin Ciocalteu's assay and aluminium chloride assay respectively [3]. *In vitro* antioxidant activity was evaluated using DPPH assay [4]. The chemicals and reagents used in present investigation were procured from S.D. Fine Chem. Ltd., Mumbai, India. The absorbance of test samples was observed using UV/VIS spectrophotometer (Schimadzu, Japan). The results have been expressed as mean \pm standard deviation (S.D).

3. Results and Discussion

The percentage yield of methanol extract of Jwarnashak Panch Kashya was found to be 14.98% w/w. The phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, steroids, triterpenoids, saponins, coumarins, glycosides, carbohydrates and proteins in the methanol extract of Jwarnashak Panch Kashya. Thus, the ethyl acetate fraction was prepared from methanol extract using standard procedures to get maximum polyphenols from methanol extract and percentage yield of ethyl acetate fraction was found to be 21.58% w/w in relation to methanol extract. Quantitative determination of total phenols and total flavonoids was done on the basis of a standard curve of gallic acid (linearity: 20 to 120 $\mu\text{g/ml}$; $r^2 = 0.9963$; figure 1) and quercetin (linearity: 25 to 150 $\mu\text{g/ml}$; $r^2 = 0.9973$; figure 2) respectively. It is evident from table 1, ethyl acetate fraction (17.99% w/w) of Jwarnashak Panch Kashya contained higher content of total phenols followed by methanol extract (9.01% w/w). Similarly, ethyl acetate fraction (9.11% w/w) of Jwarnashak Panch Kashya contained higher content of total flavonoids followed by methanol extract (5.13% w/w).

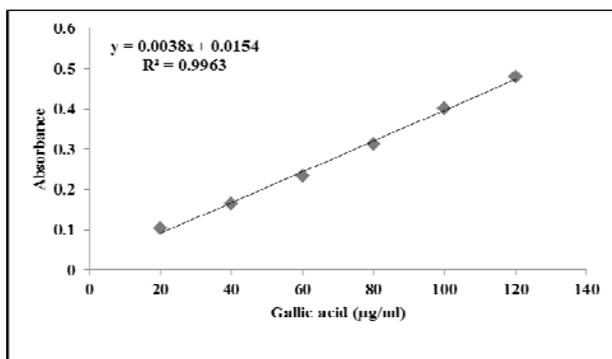


Fig 1: Standard curve of absorbance against gallic acid concentration.

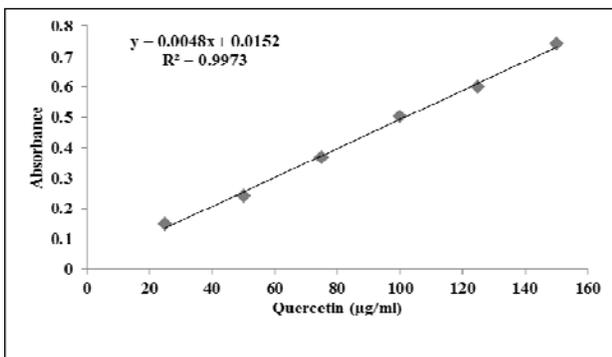


Fig. 2: Standard curve of absorbance against quercetin concentration.

Table 1: Total phenols and flavonoids content in Jwarnashak Panch Kashya.

Test sample	Total phenols content (% w/w) Mean ⁿ \pm S.D.	Total flavonoids content (% w/w) Mean ⁿ \pm S.D.
Methanol extract	9.01 \pm 1.08	5.13 \pm 0.98
Ethyl acetate fraction	17.99 \pm 1.12	9.11 \pm 0.75

n = 3

Antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. This assay is based on the measurement of the loss of DPPH free radical colour at 517 nm after reaction with test compounds and the reaction is monitored by a spectrophotometer. DPPH is a stable free radical of purple colour and in the presence of an antioxidant its colour changes to yellow based on the efficiency of the antioxidant. The change in the absorbance with respect to control (DPPH solution, 100% free radical) is calculated as percent scavenging. It is evident from table 2, methanol extract and its ethyl acetate fraction of Jwarnashak Panch Kashya showed significant activity in comparison to standard drug rutin. Ethyl acetate fraction showed maximum activity (88.25% inhibition of DPPH) at concentration of 5 $\mu\text{g/ml}$ followed by methanol extract (80.25% inhibition of DPPH) at concentration of 20 $\mu\text{g/ml}$.

Table 2: Antioxidant activity of Jwarnashak Panch Kashya.

Treatment	Concentration ($\mu\text{g/ml}$)	Mean ⁿ percent inhibition of DPPH \pm S.D.
Rutin	2	45.87 \pm 0.71
	4	79.17 \pm 0.55
	6	80.58 \pm 0.30
	8	81.17 \pm 0.01
	10	82.27 \pm 0.24
Methanol extract	5	40.25 \pm 0.58
	10	60.85 \pm 0.87
	20	80.25 \pm 0.41
	40	81.78 \pm 0.10
	80	82.87 \pm 0.14
Ethyl acetate fraction	5	88.25 \pm 0.45
	10	89.01 \pm 0.32
	20	89.91 \pm 0.78
	40	90.11 \pm 0.54
	80	91.65 \pm 0.69

n = 3

A thorough review of literature reveals that naturally occurring phenolic and flavonoidal compounds exhibit various pharmacological activities such as anticancer, antiulcer, antimutagenic, antiarthritic, antimicrobial and antioxidant. Antioxidants help to retard the risk of degenerative diseases such as cancer, brain dysfunction, arthritis, heart disease, arteriosclerosis and inflammation via inhibiting or decreasing the generation of free radicals. Preliminary phytochemical screening of polyherbal formulation showed presence of phenols and flavonoids as major classes of phytoconstituents in methanol extract of polyherbal formulation. A number of research reports showed that phenols and flavonoids have antioxidant properties such as *Actaea spicata*, *Abies pindrow*, *Calotropis gigantea*, *Coccinia indica* and *Abies webbiana* [3-6].

4. Conclusion

Finally, it is concluded that phenols and flavonoids are responsible for antioxidant activity of Jwamashak Panch Kashya.

5. Acknowledgements

Authors duly acknowledge Department of Pharmaceutical Sciences and Drug Research for providing access to instrumentation facilities at Punjabi University, Patiala.

6. References

1. Parasuraman S, Thing GS, Dhanaraj SA. Antianxiety activity of test samples exhibit a similar pattern, test values of standard samples exhibited. *Pharmacognosy Reviews*. 2014; 8(16):73-80.
2. Tripathi D. *Caraka Samhita*. Chaukhamba Surbharati Prakashan, Varanasi 2012; 3(Verse No. 197,198, 201, 202, 343)II:184,186, 218.
3. Kumar D, Jamwal A, Madaan R, Kumar S. Estimation of total phenols and flavonoids in selected Indian traditional plants. *Journal of Pharmaceutical Technology, Research and Management*. 2014; 2(1):329-338.
4. Kumar D, Jamwal A, Madaan R, Kumar S. Evaluation of antioxidant activity of selected Indian medicinal plants. *Journal of Fundamental Pharmaceutical Research*. 2014; 2(2):1-10.
5. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. *Pharmacological Reviews*. 2001; 53(2):135-159.
6. Madaan R, Bansal G, Kumar S, Sharma A. Estimation of total phenols and flavonoids in extracts of *Actaea spicata* roots and antioxidant activity studies. *Indian Journal of Pharmaceutical Sciences*. 2011; 73(2):666-669.