

PHYTOCHEMICAL STUDY AND RADICAL SCAVENGING POTENTIAL OF JOSHANDA-A HERBAL MEDICATION

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Abstract

Joshanda is a polyherbal preparation, which is used as traditional home-made remedy, to cure various contagious bronchial infections, in Unani System of Medicine, in Pakistan and South Asian region. Plants that have bioactive phyto-medicinal metabolites such as flavonoids, alkaloids, tannins and phenolics, are important to cure infections due to pharmacological effects. In Present study some selected ingredients of Joshanda i.e. *Ziziphus jujube* fruit, *Glycyrrhiza glabra* roots, *Mentha piperita* whole herb, *Althea officinalis* seeds and *Rosa indica* flowers were selected. Qualitative analysis of selected joshanda Ingredients was done to determine important phytochemicals as flavonoids, alkaloids, saponins, phenolics, tannins, triterpenes and steroids. Extraction of selected plants was done by methanol. Phytochemical analysis showed the presence of flavonoids and phenolics in all five plants selected for the study, whereas saponins were only present in *M. piperita*. Selected ingredients under study showed high DPPH radical scavenging capacity ranging from 57.02 to 94.59 %. Phenolic and flavonoids are known as antioxidants and vital bioactive compounds, which have been considered because of their human health benefits as curing many infections. The significance of our study was aimed to deliver evaluation of phyto-compounds present in selected plants.

Keywords: Antioxidant, Bronchial Infections, Flavonoids, Joshanda, Phenols, Scavenging Potential.

Introduction

Herbal medicines have therapeutic effects which are due to synergistic influence of various constituents (Sun *et al.*, 2017). According to WHO, Indigenous medicines are important resource of healthcare because its affordable cost and effectiveness and about 74 % plant-based drugs have been exposed (Gulshan *et al.*, 2012). Plant based medicines are very less toxic to human as it is observed from prolonged use by man. Climatic condition of Pakistan are appropriate for the medicinal plants that can be used for homemade remedies (Shinwari *et al.*, 2011)

and local people use these plants for their animals health care (Ismail and Nisar, 2010; Rashid and Arshad, 2002). Majority of population in rural areas depend on, agriculture and ethno botanical plants for their income resource (Said and Saeed, 1996). So, indigenous people use particular parts of plants for the formation of herbal products, for curing many diseases and from many researches it is revealed that almost 90% medicinal plants are only used by local people (Ramesh and Okigbo, 2008; Oteo *et al.*, 2005).

Many respiratory diseases such as asthma, common cold, bronchitis, cough, and pneumonia are caused due to the respiratory tract infection and due to these respiratory disorder about 2.2 million deaths occur every year (Khan *et al.*, 2014; Kayani *et al.*, 2004). Various plants belonging to different families are being used from generation to generation to treat cough, respiratory tract infection, pneumonia, and asthma, by the native population (Hameed *et al.*, 2011). Due to presence of certain phytochemicals, mostly rural population use the native plants for curing various ailments and naturally these medicinal plants have benefits for mankind (Khan *et al.*, 2014; Hameed *et al.*, 2011) because these Plants contain secondary metabolites i.e. phenols, flavonoids, tannins, steroid, alkaloid, gums and resins that are related to the antimicrobials and antibacterial activities (Aftab *et al.*, 2019; Shahid *et al.*, 2019; Asadbeigi *et al.*, 2014; Ndamane *et al.*, 2013).

Joshanda, a traditional medicinal drug which consists of almost seven ingredients and from centuries it has been used, for preparation of medicine in Unani system (Ahuja *et al.*, 2009). For flu treatment, this decoction is most popular as compared to other remedies (Hassan and Khan, 2014). In Pakistan it can be readily prepared and used against many infectious diseases, as, catarrh, respiratory infections, cold, bronchitis, cough, flu and fever and it effects smooth muscles of bronchial tissue.

This homemade formulation has many properties like antioxidant, anti-inflammatory, cytotoxic and antibacterial (Ashraf *et al.*, 2010; Khan *et al.*, 2012).

In present study particular parts of joshanda ingredients i.e. fruit of *Z. jujube*, Roots of *G. glabra*, stem and leaves of *M. piperita*, seeds of *A. officinalis* and flowers of *R. indica* were selected on the basis of their biological activity and phytochemical potential especially flavonoids and phenolics. Because these chemicals have wide-ranging biological properties which improve human health and also help to reduce the risk of many diseases related to respiratory tract. Flavonoids are important chemicals, are used as colorants, flavorants, antioxidant, antiallergic, anti-inflammatory, anticancer, anti-fungal, antimicrobial and as neuroprotective (Kumar *et al.*, 2013; Lou *et al.*, 2011). Chemical examines of jujube fruit have shown, high quantity of flavonoids, phenolics content and antioxidant activity (Pawlowska *et al.*, 2009; Kamilolu *et al.*, 2009). *G. glabra* L. is a medicinal herb found worldwide, that is a sweet wood known as 'liquorice' and it is an influential and distinctive medicinal herb (John and Stirling, 2003). British Herbal Pharmacopoeia agree that *G glabra* L. has expectorant and anti-inflammatory properties. *M. piperita* L. is a perennial, strongly aromatic herb (Sujana *et al.*, 2013). It is a potential cure for several symptoms and disorders. In many known

medicines, it is used for treatment of nausea, vomiting, flatulence, indigestion and stomach cramps. Versatile biological actions for species of *Mentha* have been observed including antibacterial effects (Oyededeji and Afolayan, 2006; Hajlaoui *et al.*, 2008). Flavonoids contents are responsible of antioxidant property of *Mentha* spp (Padamini *et al.*, 2010). *A. officinalis* is native to Europe, Asia, and USA which is conventionally used in treating oral and pharyngeal mucosa irritation, associated to dry cough, skin burns, mild gastritis and many insect bites. It is also can be used for the treatment of catarrh of the mouth, throat, gastrointestinal tract, urinary tract disorders, as well as for ulcers, inflammation, abscesses, constipation, burns and diarrhoea (SM *et al.*, 2011). It can also be used for treatment of bronchial and chest congestion, and also impart relief from a sore throat and can stop runny nose with antibacterial influence as well (Manjari *et al.*, 2011).

Materials and Methods

Selection and preparation of plant material

Joshanda ingredients “*Z. jujuba* (fruit), *G. glabra* (root), *R. indica* (flower), *A. officinalis* (seeds) and *M. piperita* (stem and leaves)” were obtained in dried form, from a traditional medicinal store and a local grocery.

Extraction

After weighing the sample, grinded the plant material into fine powder with the help of electric grinder. Crude extracts from the joshanda ingredients were obtained by dipping powder material in specific solvent as methanol for 15 to 20 days at room temperature and filtered it with the help of whatman filter paper. At room temperature filtrate was then evaporated and after that weighed the crude extract, before continuing different activates for experimental work.

Phytochemical analysis

Phytochemical analysis of selected ingredients was done for determination of important constituents present in prepared extracts via standard methods (Harborne, 1978; Harborne, 1973; Trease and Evans, 1989). For the formation of stock solutions, 10mg of every extract were dissolved in 10ml methanol to formed 1mg/ml concentration.

Alkaloids determination

For the testing of alkaloids **Dragendroff's test** was used. So, 2 ml sulphuric acid was added in 2 ml plant extract and then filtered it. After the filtration, few drops of Dragendrof;s reagent were added in solution. Orange to reddish coloration were formed which is indication of alkaloids presence.

Flavonoids determination

(a) **Alkaline Reagent test** was used to detect the flavonoids presence in sample, for this purpose 2-4 drops of (10%NaOH) were added in extract solution, yellow colour was which disappeared after addition of dilute acid.

(b) 1 ml of (5% $AlCl_3$) was added in sample solution. Appearance of yellow colour was the indication of flavonoids.

Phenolics determination

Ferric Chloride test was used to detect the phenolics presence in selected samples. In 1 ml plant extract, 2 drops of 5 % $FeCl_3$ were added. Greenish precipitation was formed which is the indication of phenolics.

Saponins determination

Distilled water (2ml) was added in sample extract (2ml) and shaken vigorously. Froth formation was the indication of saponins.

Tannins determination

Potassium dichromate test was used for detection of Tannins in sample extract. 10 % potassium dichromate (1ml) was added in extract (5ml). Yellow precipitation formation was the indication of tannins. Freshly prepared 10 % KOH (1 ml) was added in plant extract (1ml). Dirty white precipitation was the indication of tannins.

Steroids determination

In plant extract (2 ml), 5 drops of conc. H_2SO_4 was added. Red coloration was the indication of steroids.

Triterpenes determination

In plant extract (2 ml), 5 drops of conc. H_2SO_4 was added. Blue green coloration was the indication of triterpenes.

Antioxidant activity

The antioxidant activity of crude extracts of joshanda ingredients was evaluated on the basis of radical scavenging effect of the stable 1,1- diphenyl -2- picryl-hydrazyl (DPPH) free radical activity. DPPH test was used to evaluate the antioxidant activity by method in which antioxidants, act to prevent lipid oxidation, thus scavenging of DPPH free radical. The crude extracts of all samples at various concentrations (1000 $\mu g/ml$, 700 $\mu g/ml$, 500 $\mu g/ml$, 300 $\mu g/ml$, 100 $\mu g/ml$) were screened for DPPH radical scavenging activity (Charan and Gupta, 2013). About 1.3 unit of absorbance was observed when DPPH methanol solution (0.125 mg/mL) was retained at 517nm. DPPH 0.125 mg/ml was formed by dissolving DPPH (5 mg) in methanol (200ml). Firstly, all extracts were selected at 1000 $\mu g/mL$ concentration for their antiradical ability and then screened at other various concentrations of 700 $\mu g/mL$, 500 $\mu g/mL$, 300 $\mu g/mL$ and 100 $\mu g/mL$. In each sample DPPH solution (1.4 ml) was added and samples were incubated at room

temperature for 30 min. Ascorbic acid acts as antioxidant, which can protect cells from damage as the result of free radicals. (Ascorbic acid 12 mg/ml + DPPH), methanol and (DPPH + Distilled water) were used as standard, blank and control respectively. At 517 nm absorbance were recorded of each sample. Following formula was used to determine Radical scavenging activity:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_s)/A_0 \times 100]$$

In above formula A_0 is an absorbance of control with methanol and A_s is an absorbance of each sample extract.

Results and Discussion

Table 1: Percentage yield of the dry weight of (Methanolic) extracts

Plant material	Dry weight (g)	Methanol extract (g)	% yield of dry Weight
<i>Z. jujube</i>	50	8.34	16.68
<i>G. glabra</i>	50	1.44	2.88
<i>M. piperita</i>	50	2.51	5.02
<i>A. officinalis</i>	50	1.24	2.48
<i>R. indica</i>	50	2.52	5.04

Table 2: Phytochemical analysis of crude extract of joshanda ingredients

Plants	Flav	Alk	Phenol	Sap	St	Tann	Trit
<i>Z. jujube</i>	+	+	+	-	+	+	+
<i>G. glabra</i>	++	+	+	-	+	-	-
<i>M. piperita</i>	++	+	+++	+	-	+++	+++
<i>A. officinalis</i>	+	-	+++	-	+	++	-
<i>R. indica</i>	+	-	+++	-	-	+++	-

+++ Intense coloration or ppt., ++ Moderate, + Extremely light, -- Not detected, **Flav**= flavonoids, **Alk**= alkaloids, **Phenol**= phenolics, **Sap**= saponins, **St**= steroids, **Tann**= tannins, **Trit**= triterpenoids.

Crude extract preparation

In present study crude extract of joshanda ingredient was prepared for further experiment as shown in **Table 1** the % yield of dry weight of methanolic crude extracts of *Z. jujube*, *G. glabra*, *M. piperita*, *A. officinalis* and *R. indica* was 16.68, 2.88, 5.02, 2.48 and 5.04 respectively i.e. *Z. jujube* > *R. indica* > *M. piperita* > *G. glabra* > *A. officinalis*.

Qualitative analysis

Phytochemical analysis was performed to identify active components in of selected ingredients. Results of qualitative analysis showed by **table 2** and it is cleared by figure 1-5.

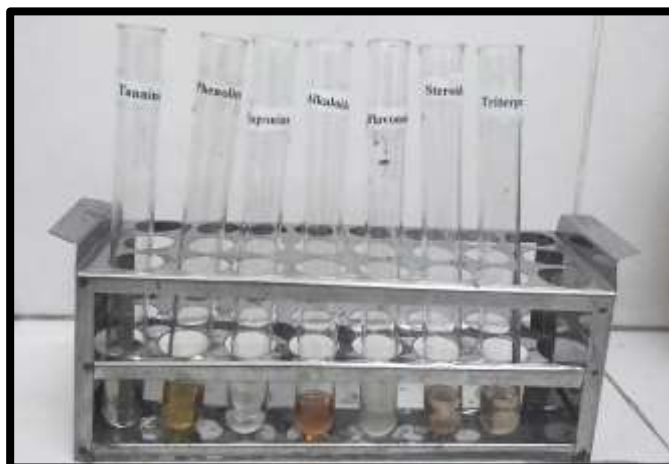


Figure 1: Qualitative analysis of *Z. jujuba*



Figure 2: Qualitative analysis of *G. glabra*

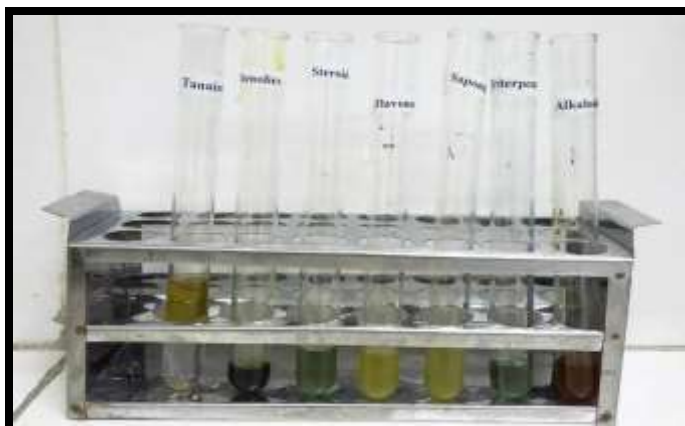


Figure 3: Qualitative analysis of *M. piperita*



Figure 4: Qualitative analysis of *A. officinalis*

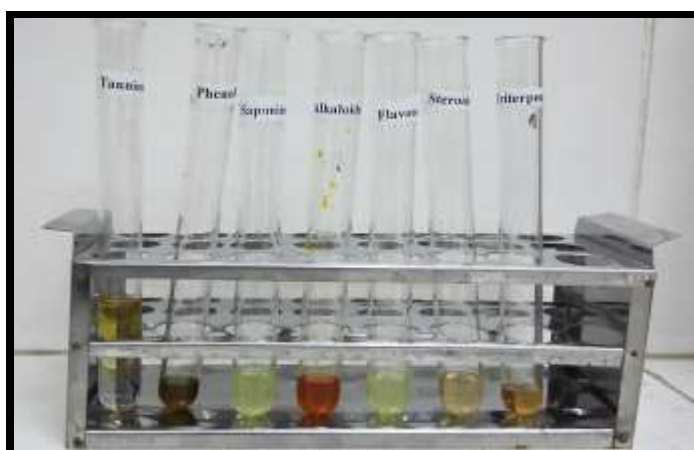


Figure 5: Qualitative analysis of *R. indica*

Table 3: DPPH radical scavenging capacity of methanolic crude extracts of joshanda ingredients

Plants	Concentration of sample (µg/ml)	Absorbance				Scavenging
		A ₁	A ₂	A ₃	Mean±S.D	%
Ascorbic acid	100	0.029	0.028	0.027	0.028±0.00081	92.43
	300	0.025	0.025	0.025	0.025±0	93.24
	500	0.017	0.015	0.017	0.016±0.00094	95.67
	700	0.009	0.009	0.010	0.01±0.00081	97.29
	1000	0.005	0.004	0.005	0.004±0.00047	98.91
<i>Z. jujuba</i>	100	0.038	0.039	0.037	0.038±0.00081	89.73
	300	0.022	0.023	0.023	0.022±0.00047	93.78
	500	0.021	0.020	0.019	0.022±0.00081	94.59
	700	0.020	0.020	0.020	0.022±0	94.59
	1000	0.035	0.034	0.035	0.034±0.00047	90.54
<i>G. glabra</i>	100	0.157	0.159	0.158	0.158±0.00081	57.02
	300	0.145	0.146	0.147	0.146±0.00081	60.54
	500	0.101	0.100	0.102	0.101±0.00081	72.43
	700	0.064	0.063	0.062	0.063±0.00081	82.7
	1000	0.056	0.057	0.058	0.057±0.00081	84.59
<i>M. piperita</i>	100	0.119	0.118	0.120	0.119±0.00081	67.84
	300	0.088	0.089	0.090	0.089±0.00081	75.94
	500	0.075	0.075	0.075	0.075±0	79.73
	700	0.074	0.073	0.075	0.074±0.00081	80
	1000	0.044	0.044	0.044	0.044±0	88.1
<i>A. officinalis</i>	100	0.091	0.092	0.093	0.092±0.00081	75.13
	300	0.038	0.039	0.04	0.039±0.00081	89.45
	500	0.069	0.069	0.069	0.069±0	81.35
	700	0.043	0.042	0.042	0.042±0.00047	88.37
	1000	0.041	0.040	0.039	0.04±0.00081	89.18
<i>R. indica</i>	100	0.073	0.073	0.073	0.073±0	80.27
	300	0.073	0.072	0.071	0.072±0.00081	80.54
	500	0.052	0.053	0.051	0.052±0.00081	85.67
	700	0.052	0.050	0.051	0.051±0.00081	86.48
	1000	0.040	0.041	0.042	0.041±0.00081	89

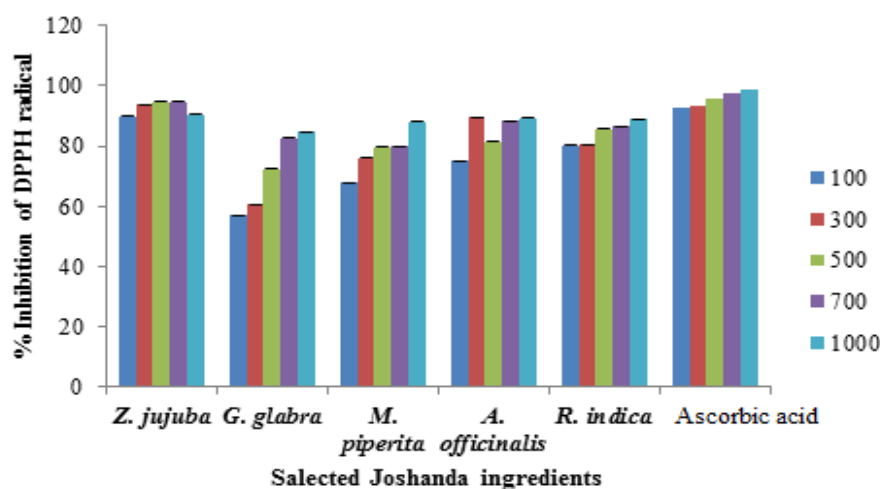


Figure 6: Comparison between % inhibitions of DPPH radical at different concentrations in selected joshanda ingredients

The crude methanol extracts of joshanda ingredients (1mg/ml) were analysed for free radical scavenging capacity through DPPH radical scavenging method. And extracts were used to evaluate their free radical scavenging capacity at further concentrations as 100, 700, 500, 300 and 100 μ g/ml. All joshanda ingredients have high DPPH radical scavenging capacity as shows in (Table 3) ranges from 57.02 to 94.59 %.

Comparison between % inhibitions of DPPH radical at different concentration in selected joshanda ingredients were shows in (Figure 6). Extracts obtained from all the five plants were found to be highly antioxidative in nature. So IC_{50} was not possible to calculate because inhibition % age was in the range of 57.02 to 94.59 %. Ascorbic acid was taken as standard and shows maximum % inhibition scavenging.

Herbs since time immemorial have been in use being remedies for various diseases inclusive of contagious types. Common cold treated with success using a combination of individual herbs collectively known as Joshanda in Unani System of Medicine in South Asian region. The clinical picture of common cold is manifestation of biological activities of a collection of both viruses and bacteria (Azmi *et al.*, 2010). Present study primarily aimed at the identification and

quantification of flavonoids and phenolics in selected joshanda ingredients that are used for bronchitis diseases. It is quite evident from results that all the selected plants were rich in flavonoids and phenolics in addition to other secondary metabolites. These two groups of phytochemicals were common in all plants selected for the study (Tungmunnithum *et al.*, 2018). Among all selected ingredients of joshanda, saponins were only present in *M. piperita* as reported by (Adham, 2018).

Antioxidants have the capacity to inhibit the oxidation of other particle ingredients that have high free radical scavenging ability. Similar results have been reported by (Charan and Gupta, 2013; Chopra *et al.*, 2013). Different studies recommend that various biological actions of flavonoids and phenolic are related to their antioxidant action. The antioxidant property of Joshanda can be due to the presence of phenolic acids and flavonoids, which in turn exert this action due to the presence of free hydroxyls (Cai *et al.*, 2006). Various studies reported that medicinal plants act as eventual core of natural antioxidant chemical compounds, predominantly secondary metabolites of plants as phenolic and flavonoids that are produced by plant naturally, to protect itself under unfavourable environments. These both compounds are generally known as the

major phytochemicals with antioxidant abilities (Tungmunnithum *et al.*, 2018).

Conclusion

From recent studies it is clear that flavonoids and phenolics have properties of anti-inflammatory and anti-oxidant. The phytochemical analysis of percentage crude yields of chemical components of selected plants revealed the presence of flavonoids and phenolics.

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