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Qualitative and quantitative phytochemical screening of *in vivo* and *in vitro* extracts of *Fagonia schweinfurthii* hadidi- a potential medicinal plant species of western Rajasthan

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ABSTRACT

Fagonia schweinfurthii H. commonly identifid as Dhamasa, holds significance as an important medicinal species within the Zygophyllaceae family. They populate dry, desert areas all around the globe. It is distributed in different states of India, including Rajasthan, Gujarat, Haryana, Punjab, Maharashtra, Karnataka, and Tamil-Nadu. The present work aimed to identify and analyze the bioactive compounds, total flavonoid and phenolic content of *in vivo* plant parts (stem and leaf) and *in vitro* derived callus of *F. schweinfurthii* H. in petroleum ether, chloroform, acetone, ethanol, methanol and aqueous extracts by spectrophotometric method. Alkaloids, saponins, flavonoids, phytosterols, cardiac glycosides, triterpenoids, tannins and phenolic compounds were observed in the preliminary phytochemical analysis. Total flavonoid and phenolic content in different solvent extracts were determined as compared to standard quercetin and gallic acid, respectively. Both the highest concentration of flavonoids (40.33±0.88) mg QE/g and phenol (93.54±0.26) mg GAE/g was found in methanolic extract of callus. The presence of these biologically active substances indicates the therapeutic significance of the plant. Experimental results indicated that *in vitro* derived callus of *F. schweinfurthii* H. could be a promising reservoir of bioactive compounds to be utilized on an industrial scale for therapeutic purposes.

Key words: *Fagonia schweinfurthii H., Phytochemical screening, Total phenolic content, Total flavonoid content, In vitro extract, Medicinal plant*

Introduction

Fagonia schweinfurthii H. a small spiny under shrub of Zygophyllaceae family, is a potential medicinal plant species found in western Rajasthan. Dhamaso/Dhamasa is the local name of this plant. They are found in arid desert regions worldwide (Bhandari, 1990; Pareek *et al*., 2012; Naik, 2016). In Ayurveda and folkloric literature, it is widely recognized as Dhamasa, Dharulabha, Dhanvayasa, Samudranta, Dusparsha, etc. (Bhandari, 1990; Naik, 2016). Traditional practitioners have ethnobotanically utilized *Fagonia* species under Ayurveda and other healing regimes for many diseases (Singh and Pandey, 1998; Puri and Bhandari, 2014). This plant demonstrates a spectrum of medicinal properties, including antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, analgesic, anti-

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pyretic, prophylactic activity against small pox agent and antihistaminic properties. It is used to treat diabetes, joint pain, fever, cough, asthma, pain relief, ear infection, urinary discharge, blood purifier, stomach ache, wound healing, skin and venereal disease (Rathore et al., 2011; Pareek and Nagori, 2013; Puri *et al.,* 2015). Plant harbors medicinal attributes because of their bioactive phytochemical constituents that have specific physiological effect on humans. This medicinally important plant species is known to contain phytochemicals especially alkaloids, terpenoids, flavonoids, saponins, phytosterols, tannins, cardiac glycosides, mucilage and trace elements (Rathore *et al.*, 2011; Puri and Bhandari, 2014).

To our knowledge, no studies have been published comparing the phytochemical analysis of different solvent extracts of *in vitro* derived callus and *in vivo* plant parts (stem and leaves) of this medicinal plant species. Therefore, the current study's objectives were to evaluate the total flavonoid and phenolic content across different solvent extracts of *in vitro*-derived callus and *in vivo* plant parts (stem and leaves) and also preliminary phytochemical screening of the same.

Materials and Methods

Collection of plant sample and extract preparation

The fresh, healthy and disease- free plant material of *F. schweinfurthii* H. was collected from Karni Nagar, Jodhpur, Rajasthan and was identified and verified by BSI (Botanical Survey of India) Jodhpur, Rajasthan. The fresh plant material was first rinsed under running water to remove dust and other extraneous fine particles. *In vivo* plant parts (leaves and stem) and *in vitro* derived callus were dried at room temperature under shade. After drying, plant samples were powdered separately using a mechanical grinder into a fine powder. The dried, powdered plant materials were subjected to successive extraction in a soxhlet apparatus, using a spectrum of solvents ranging from non polar to polar including petroleum ether, chloroform, acetone, ethanol, methanol and water. The solvent containing plant extracts were filtered and placed in water bath for evaporation of excess solvent, after which the dry extracts were kept in air tight containers for further examination.

Preliminary qualitative screening of phytochemical

Preliminary qualitative screening of phyto-chemicals was conducted for the identification of bioactive compounds including alkaloids, saponins, flavonoids, phytosterols, cardiac glycosides, triterpenoids, tannins and phenolic compounds in different *in vitro* (callus) and *in vivo* (leaves and stem) plant extracts by using physico-chemical analysis test (Kokate,1984; Raman, 2006; Shaikh and Patil, 2020). All the extracts were subjected for phytochemical screening by dissolving them in respective solvent (1 mg/ml) and phyto-chemicals were detected by using different chemical reagents Table 1.

Total phenolic content

The determination of total phenolic content in the plant extracts was conducted, employing Folin-Ciocalteu's reagent (FCR) incorporating slight modifications (Singleton *et al.,* 1999). 500 microlitre of plant extract (1 mg/ml) was made upto one ml with distilled water, mixed thoroughly with 2.5 ml of FC reagent (dilution ratio of 1:10,V/V) and maintained at ambient temperature for a period of five minutes. The reaction mixture was given a 90 minutes dark incubation period at room temperature after 1.5 ml of sodium carbonate (7.5%, W/V) was added. At 765 nm, absorbance of reaction mixture was measured. The same protocol was replicated for standard gallic acid (10-100 µg/ml) solution and total phenolic content in the different solvent extract was accomplished using standard curve based on gallic acid equivalent (GAE). The total phenol content was represented as mg of gallic acid equivalents (GAE) per gram of dry extract.

Total flavonoid content

The determination of total flavonoid content was carried out employing the colorimetric method reported by Zhishen *et al.,* 1999. 500 microlitre of plant extract (1 mg/ml) was brought upto a final volume of one ml using distilled water. Subsequently, 150 microlitre of 5% $NaNO_2$ solution was added and incubated for five minutes. The reaction mixture was allowed to incubate at ambient temperature for a period of six minutes following the addition of 150 microlitre of $AlCl_3$ (10%, W/V) solution. Then, two ml of 4% NaOH solution was added and made upto five ml with distilled water. The reaction mixture

Table 1.

S. No.	Name of phytochemical	Name of test	Test procedure	Appearance
1.	Alkaloids	Each solvent free extract was given a brief stir with a few millilitres of dilute HCl before being filtered. Tests were conducted on the filtrate using several alkaloidal reagents as mentioned below-		
		Dragendorff's test	One to two ml of Dragendorff's reagent mixed with a few ml of filtrate	Presence of prominent yellow/ orange precipitate
		Wagner's test	One to two ml of filtrate + Wagner's reagent (few drops)	Presence of reddish- brown precipitate
		Mayer's test	Few ml of filtrate + Mayer's reagent (one to two drops)	Presence of creamy or white precipitate
		Hager's test	Few ml of filtrate + one or two ml of Hager's reagent	Presence of yellowish precipitate
2.	Flavonoids	Lead acetate test	Two to three ml of extract mixed with few ml of lead acetate solution (10%)	Presence of yellowish precipitate
		Alkaline reagent test	Few ml of extract + few ml of 10% ammonium hydroxide solution	Presence of intense yellow colour
		Ferric chloride test	Few ml of plant extract + few ml of 10% ferric chloride solution	Presence of intense green colour
3.	Phytosterols	Libermann- Burchard's test	Fifty mg of plant extract dissolved in two ml of acetic anhydride + two to three drops of concentrated H_2SO_4 (around the tube's edge)	An array of colour changes
		Salkowski's test	Few ml of extract + few ml of chloroform + two to three drops of concentrated H_2SO_4 (firmly shaken and allow to settle)	Lower layer exhibit reddish colour
4.	Saponins	Foam test	25 mg extract + 10 ml distilled water + shaken vigorously	Persistent froth
5.	Cardiac glycosides	Legal's test	Few ml of alcoholic extract + one ml of pyridine and sodium nitroprusside + 10% NaOH	Presence of pink to red colour
		Keller-killani test	One ml of extract+ 1.5 ml of glacial acetic acid + 5% solution of $FeCl_3$ (single drop) + concentrated H_2SO_4 (along the test tube's side)	Acetic acid layer contains blue colour
6.	Phenolic compounds and Tannins	Gelatin test	50 mg of plant extract + Five ml of distilled water + two ml solution, comprising 1% gelatin, enriched with 10% sodium chloride	Presence of whitish precipitate
		Lead acetate test	50 mg of extract + three ml s of 10% lead acetate solution	Presence of bulky white precipitate

			and few ml of distilled water	
		Ferric chloride test	50 mg of plant extract + Five ml of distilled water + two to three drops of solution, comprising 5% $FeCl_3$, containing 10% NaCl	Presence of dark green colour
		Alkaline reagent test	10% of ammonium hydroxide solution mixed with plant extract (aqueous solution)	Presence of yellow fluorescence
7.	Anthraquinone	Borntrager's test	2 ml of hydrolysate filterate (50 mg of extract + two ml of dilute hydrochloric acid + 10 min. boil in water bath and filter) + three ml of chloroform (shaken gently)+ separation of chloroform layer+ 10% ammonia solution	Presence of pink colour
8	Triterpenoids	Slkowski's test	Filtrate (extract dissolved in chloroform) + two to three drops of concentrated H_2SO_4 (vigorously shaken and let to settle)	Golden yellow layer present (at the bottom)

was completely mixed and then incubated for 15 minutes at room temperature. At 510 nm, absorbance of reaction mixture was measured. The emergence of pink colour suggested the presence of flavonoids. The same process was repeated using standard quercetin (10-100 µg/ml) solution and the standard curve of quercetin equivalent (QE) was used to determine the total flavonoid content of plant extract. The amount of flavonoids present in the total was represented as mg of quercetin equivalent (QE) per gram of dry extract.

Results and Discussion

Preliminary qualitative phytochemical screening

Since ancient times, plants have been utilized for medicinal purposes, so it is important to screen the plant to know its active principle constituents. The examination of phyto-chemicals revealed the existence of secondary metabolites with biological significance. Consequently, it is essential to evaluate the qualitative and quantitative phytochemical assessment of *F. schweinfurthii* H. The current study revealed that the different solvent extracts of *in vitro* derived callus and *in vivo* plant parts (leaves and stem) of *F. schweinfurthii* H. contained alkaloids, flavonoids, phytosterols, saponins, cardiac glycosides, tannins, triterpenoids and phenolic compounds. Table 2 shows the phytochemical screening of leaf, stem and callus in different solvent extracts of plant. Ethanolic, methanolic and aqueous extract of callus and leaf contained higher number of secondary metabolites than any other solvent extracts. However, methanolic callus extract showed high degree of precipitation. Similar to our findings, Sharma *et al.* (2013) and Naik (2016) also reported the presence of alkaloids, tannins, flavonoids, steroids, saponins, terpenoids and cardiac glycosides in whole plant of *F. sweinfurthii* H. Moreover, Pareek *et al.* (2012) reported the presence of flavonoids, tannins, alkaloids, saponins, steroids and cardiac glycosides in stem, leaf, flower and fruit extract of *F. indica.* Rathore *et al.* (2011) evaluated root powder of *F. schweinfurthii* H. for its phytochemical status. Phytochemical screening in various non-polar and polar solvent extracts of *Fagonia cretica* has been reviewed earlier also (Sajid *et al.*, 2011; Eman, 2011; Dastagir *et al.*, 2012; Qureshi *et al.*, 2016). They reported that the most active fraction was found to be the crude methanolic extract of entire plant material during their qualitative phytochemical screening. This is most likely caused by methanol's comparatively better capacity as a solvent for phytochemicals exraction (Qureshi *et al.*, 2016).

Total phenolic content

As secondary metabolites, phenolic compounds are commonly produced by plants and they are acknowledged for their antioxidant properties. Folin-Ciocalteau reagent was used to quantify TPC and estimation was done using gallic acid as a reference.

Table 2. Screening of phytochemical constituents of various extracts from stem, leaf and callus of *F. sweinfurthii* H. through soxhlet extraction method

Phytoconstituents → Solvents ↓	Plant parts	Alkaloids	Flavonoids	Phenolic compound and tannins	Saponins	Phytosterol	Cardiac glycosides	Anthra-quinone	Triter-penoids
Petroleum Ether	LE	-	-	-	-	++	-	-	-
	SE	-	-	-	-	+	-	-	-
	CE	-	-	-	-	++	-	-	-
Chloroform	LE	+	+	-	-	++	-	-	-
	SE	-	+	-	-	+	-	-	-
	CE	+	+	-	-	++	+	-	-
Acetone	LE	+	+	++	-	+	-	-	-
	SE	+	+	+	-	+	-	-	-
	CE	+	+	++	-	+	+	-	-
Ethanol	LE	++	+++	++	+	-	+	-	+
	SE	++	++	+	-	-	-	-	+
	CE	++	+++	++	+	-	+	-	+
Methanol	LE	++	+++	+++	+++	-	+	-	+
	SE	++	++	++	++	-	-	-	+
	CE	+++	+++	+++	+++	-	++	-	++
Water	LE	+	++	++	++	-	+	-	+
	SE	+	-	+	+	-	-	-	+
	CE	+	++	++	+	-	+	-	+

LE- Leaf extract, SE- Stem extract, CE- Callus extract
(+++: highly present, ++: moderately, +: low, - : absent)

Table 3. Total phenolic content in stem, leaves and *in vitro* produced callus of *F. schweinfurthii* H.

S. No.	Solvent	Total phenolic content (mg GAE/g) Stem	Leaf	Callus
1.	Petroleum ether	17.55±0.42[a]	19.38±0.41[a]	18.33±0.33[a]
2.	Chloroform	41.11±0.67[b]	42.38±0.19[b]	42.75±0.88[b]
3.	Acetone	57.60±0.42[c]	59.05±0.25[c]	60.72±0.25[c]
4.	Ethanol	63.55±0.09[d]	61.88±0.09[d]	62.88±0.25[d]
5.	Methanol	86.44±0.67[f]	89.16±0.28[f]	93.54±0.26[f]
6.	Water	64.88±0.25[e]	65.83±0.33[e]	67.16±0.29[e]

Value (mean± SD of three replicates) with different letters indicating significant differences at a significance level of $P<0.05$.

Methanolic callus extract showed highest phenolic content (93.54±0.26 mg GAE/g), however the stem's petroleum ether extract showed lowest phenolic content (17.55±0.42 mg GAE/g) (Table 3, Fig. 1). Pareek and Nagori (2013) reported the presence of highest total phenol content in methanolic extract of whole plant of *F. schweinfurthii* H. However, Rehman *et al.* (2019) reported highest total phenolic content in chloroform extract derived from *Fagonia indica*'s aerial parts. An *in vitro* antioxidant profiling of solvent extracts from various parts of *Fagonia cretica* for comparative analysis was reported by Iqbal *et al.* (2014). Additionally, they also observed that the methanolic root extract of *Fagonia cretica* contained the highest level of total phenol content.

Total flavonoid content

Naturally occurring antioxidants, flavonoids are essential to plants defense system. In addition, they have strong anti-inflammatory, anti-allergic and anti-cancer effects and are easily assimilated by the human body (Crozier *et al*., 2006; Rebaya *et al*., 2014). Aluminium chloride colorometric method was used to quantify TFC and estimation was done using quercetin as a reference. Methanolic callus extract showed highest flavonoid content (40.33±0.88) mg QE/g, however, petroleum ether extract of stem showed lowest flavonoid content (10.22± 0.19) mg

Table 4. Total flavonoid content in stem, leaves and *in vitro* produced callus of *F. schweinfurthii* H.

S. No.	Solvent	Total flavonoid content (mg QE/g)		
		Stem	Leaf	Callus
1.	Petroleum ether	10.22±0.19[a]	14.55±0.38[a]	12.55±0.50[a]
2.	Chloroform	17.22±0.69[b]	19.10±0.50[b]	20.55±0.50[b]
3.	Acetone	21.33±0.88[c]	24.55±0.69[c]	25.21±0.50[c]
4.	Ethanol	25.21±0.50[d]	25.44±0.19[d]	26.55±0.50[d]
5.	Methanol	31.33±0.33[f]	33.77±0.50[f]	40.33±0.88[f]
6.	Water	27.55±0.19[e]	28.77±0.19[e]	30.66±0.33[e]

Value (mean± SD of three replicates) with different letters indicating significant differences at a significance level of $P<0.05$.

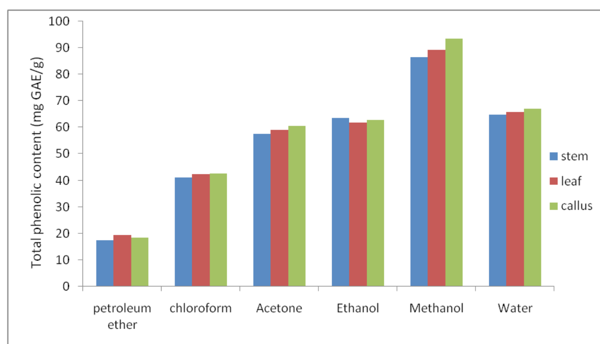

Fig. 1. Comparison of total phenolic content of *in vivo* plant parts (stem and leaf) and *in vitro* derived callus of *F. schweinfurthii* H.

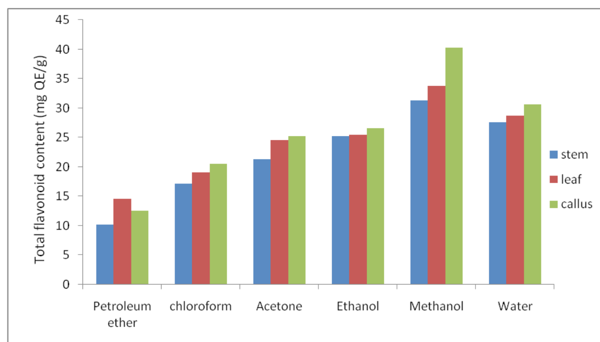

Fig. 2. Comparison of total flavonoid content of *in vivo* plant parts (stem and leaf) and *in vitro* derived callus of *F. schweinfurthii* H.

QE/g (Table 4, Fig. 2). Pareek and Nagori (2013) reported the presence of highest TFC (total flavonoid content) in methanolic extract of whole plant of *F. schweinfurthii* H. However, Rehman *et al*. (2019) reported highest total flavonoid content in chloroform extract derived from the aerial portions of *Fagonia indica* Burm F. Moreover, a study conducted by Iqbal *et al.* (2014) evaluating the *in vitro* antioxidant profiling of solvent extracts derived from various parts of *Fagonia cretica* revealed that the methanolic root extract of plant had the highest total amount of flavonoids.

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Conflict of interest

Authors declare no conflict of interest.

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