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Phytochemical Analysis of *Fagonia schweinfurthii* Hadidi

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Authors' contributions

This work was carried out in collaboration between both authors. Author YJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author BKJ managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Fagonia schweinfurthii Hadidi (family zygophyllaceae) is plant of desert region .In Gujarat it is found on the coastal area of Dwarka, Bet Dwarka and Rann of Kachcha. It is locally called dhamasia or dhamaso. Plants parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds which can be employed in the control or treatment of infectious disease which causes damage to the respiratory system, urinary tract, gastrointestinal and bilary systems and on the skin. The plant parts contain chemical components that are medically active. Tribal people living in desert region use this plant to cure number of ailments such as skin eruptions, skin diseases, antipyretic, in pain relief, in heal sores, ear infection, venereal diseases etc. In the new era of science and technology there is increasing need to validate the claims of traditional knowledge database for safe, easily available, cheap, side- effect free healthcare provisions. In the present study preliminary phytochemical analysis of plant parts of Fagonia schweinfurthii Hadidi was done by the standard method of Sofowora, 1982; Trease & Evans, 1989 method, which confirms the presence of alkaloids, tannin, saponin, terpinoid and steroids. High Performance Thin Layer Chromatography method was developed using mobile phase containing Chloroform: methanol: acetic acid in ratio 70:30:0.2 for detecting phyto-active constituents from the methanolic extracts of the leaf, stem and root of Fagonia schweinfurthii Hadidi. Methanolic extract of Fagonia schweinfurthii Hadidi of leaf, stem, fruit and root detected 8, 8, 7 and 4 numbers of peaks at 254 nm and at 366 nm 10, 12, 13 and 12 numbers of peaks from leaf, stem, fruit and root extract of Fagonia schweinfurthii Hadidi were detected.

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1. INTRODUCTION

Fagonia schweinfurthii (Hadidi) belongs to family Zygophyllaceae and found as a shrub in Western India, upper Gangetic plains and Peninsular India. Fagonia arabica Linn. is synonym of F. schweinfurthii Hadidi. In Ayurveda it is called as Dhanvayaasa, Dhanvayavaasa, Dhanvayaasaka, Samudraantaa, Duraalabhaa, Gaandhaari, Kachhuraa, Anantaa, Duhsparshaa and it is locally known as Dhramau, Dhamaso, Kandhera, dhamasa and dhamasia. The plant parts contain chemical components that are medically active. Tribal people living in desert region use this plant to cure number of ailments such as skin eruptions, skin diseases and anti- pyretic, heal sores, ear infection, venereal diseases etc [1]. In the new era of science and technology there is increasing need to validate the claims of traditional knowledge database for safe, easily available, cheap, side- effect free healthcare provisions. Plant produces a wide varieties of phytochemicals which are responsible for protecting the plant against environmental hazards such as pollution, stress, drought, UV exposure, pathogenic attack, microbial infections [2]. The phytochemicals produced by plant are categorized in to:1) primary metabolites for plants and 2)Secondary producing food metabolites for growth and protection of plants.

Standardization of plant materials is a need now pharmacopoeia Several davs. containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. The studies on phytochemical analysis have attracted the plant scientists due to its potential to cure diseases and development of new biotechnological tools and techniques. This biotechnological tool played a major role in giving the explanation to efficient problems on the one hand and provides the supplementary resources of raw material for pharmaceutical industry on the other hand. The potential of chemotaxonomy is now become increasingly obvious because of improvement of natural product chemistry. The application of chemical data to methodical has received attention of a large number of biochemists and botanists during the last three decades [3]. Preliminary screening of plant parts of Fagonia schweinfurthii reveals the presence of phytoconstituents' in it but cannot predict the maximum number of compounds in the plant parts which influence the plants medicinal properties. Hence, to determine the number of compounds HPTLC technique was used.

Chromatographic fingerprinting techniques are most significant methods which can be used for the routine herbal drug analysis and for quality assurance. HPTLC offers better resolution and estimation of active constituents with reasonable accuracy in a shorter time [4]. Pharmaceutical industry is developing very fast by analyzing phytochemicals from medicinal plants. Farmers remove some plants for their cash crops yield, although these plants have medicinal properties. The plants regularly removed from field. In the present study attempts were made to study of Phytochemistry of various phyto constituents of leaf, stem, fruit and root of *Fagonia schweinfurthii* (Hadidi) using Wagner's test and HPTLC.

2. MATERIALS AND METHODS

2.1 Preliminary Observation

Chemical tests were carried out using aqueous extracts of plant parts of *Fagonia schweinfurthii* Hadidi to identify various phyto-constitutes using standard methods [5,6,7].

2.1.1Sample preparation for phytochemical screening

50 gm powdered sample was weighed and taken separately. The powder was moistened with ammonia and evaporated to dryness. Dried sample was extracted with chloroform and filtered. After filtration, 10% sulfuric acid was added to the filtrate using separating funnel and aqueous layer was separated by adjusting pH 8 with ammonia. After adjusting pH of extract the solution with chloroform and the organic extract which obtained is evaporated to concentrate by keeping in open at room temperature. However, aqueous extraction was evaporated to dryness by heating in water-bath to obtain semi solid mass. Dried extract was stored in refrigerator for their future use in phytochemical analysis. Dried extract was re- dissolved by adding 5ml of distilled water.

2.2 Test for Alkaloid

3 ml aqueous extract was stirred with 3 ml of 1% HCl on steam bath. Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

2.3 Test for Flavonoids

To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

2.3.1 Tests for steroids

A red color produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it. This indicates the presence of steroids. Development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

2.3.2 Preparation of extracts for HPTLC

1 g of plant parts of leaf, stem, fruit and root of *Fagonia schweinfurthii* Hadidi was taken and reflux with 250 ml of methanol using a soxhlet apparatus on a water bath for 60 minutes for two cycles. Filter the extract and concentrate to 5 ml then the sample extract obtained so far is used for further analysis. Then the extract was filtered with Whatman filter paper No 1. The filtrates were stored in a glass bottle in the freeze at 8°c temperature [8].

3. RESULTS AND DISCUSSION

3.1 Preliminary Screening

The presence of alkaloids was confirmed in all parts of *Fagonia schweinfurthii* Hadidi by using Wagner's test. Flavonoids and Steroids were present in all parts of *Fagonia schweinfurthii*.

3.2 HPTLC (High Performance Thin Layer Chromatography)

3.2.1 Determination of Phyto-constitutes by HPTLC

For HPTLC aluminium plate support silica gel60F254, 10X100 cm (Merck) plates were cut with ordinary household scissors and markings were made with soft pencil. Silica gel plates were impregnated by dipping to in methanol followed by drying at room temperature for 1 hr. Sample

was applied using Band wise with Linomat 5 (camaq. muttez: Switzerland) sprav on automated instrument for HPTLC. Applied sample band length 6.9 mm 8 tracks, track distance 10.7mm, distance from lower edge 15mm; application volume 10 and 20µl of sample at 8 tracks. Camag twin through chamber with Chloroform: methanol: acetic acid (70:30:0.2, v/v/v) after20 min pre-saturation with mobile phase for development were used for plant parts of Fagonia schweinfurthii Hadidi (API vol 5). The eiaht developments over 75 mm with intermediate drying after the run plate were dried with the help of Air Dryer for detection of active compound. The camag TLC Scanner 3 by win CATS controlled software was used for densitometry analysis. For this densitometry analysis observed Absorption measurement at 254 and 366 with TLC Scanner controlled by win CATS 3 software. Retention factors are calculated by the densitometer [9].

The *R*f value indicated the position at which a substance is located in the chromatogram. *R*f values were calculated using the following formula: The *R*f values of respective compounds for all the chromatograms are depicted in Table 1 and Table 2 which showed number of compounds present in that particular class of phytoconstituents.

Retention time (Rf) = Distance travelled by the solute/distance travelled by the solvent.

The mobile phase of API volume 5 was applied on *Fagonia schweinfurthii*. Methanolic extract of *Fagonia schweinfurthii* of leaf, stem, fruit and root extracts of 20 μ l were taken. *Fagonia schweinfurthii* leaf, stem, fruit and root the ratio of chloroform: methanol: acetic acid (70:30:0.2) was used as mobile phase.

Fagonia schweinfurthii leaf methanolic extract of volume 20ul at 254nm detected 8 peaks with Rf values 0.01, 0.04, 0.08, 0.18, 0.36, 0.42, 0.58 and 0.71 were calculated. Stem methanol extract detected 8 peaks with Rf values 0.01, 0.07, 0.11, 0.15, 0.34, 0.40, 0.51 and 0.72. Fruit methanol extract detected 7 peaks with Rf values are 0.03, 0.09, 0.16, 0.35, 0.45, 0.56 and 0.72. Root methanol extract detected 4 peaks with Rf values are 0.03, 0.15, 0.38 and 0.40 respectively. (Table 2, Fig. 1) The present study of HPTLC reveals that methanolic leaf extract and stem extract of Fagonia schweinfurthii at 254nm separates 8 compounds, respectively. Fruit methanolic

extract separated 7 compounds, while root methanol extract separated 4 compounds. All the components separated from the plant parts are unknown except from fruit extract at peak number 2 at Rf 0.09.

Fagonia schweinfurthii leaf methanolic extract of 20µl at 366nm detected 10 peaks with R_f values 0.02, 0.05, 0.08, 0.12, 0.18, 0.36, 0.42, 0.46, 0.59 and 0.86 were calculated. Stem methanol extract detected 12 peaks with R_f values 0.03, 0.07, 0.15, 0.25, 0.34, 0.40, 0.48, 0.51, 0.46,

0.64. 0.67 and 0.86. Fruit methanol extract detected 13 peaks with R_f values 0.01, 0.06, 0.16, 0.35, 0.41, 0.44, 0.61, 0.64, 0.72, 0.80, 0.83, 0.86 and 0.98. Root methanol extract detected 12 peaks their R_f values are 0.16, 0.23, 0.26, 0.36, 0.38, 0.52, 0.55, 0.69, 0.72, 0.83, 0.85 and 0.89 respectively. (Table 3, Fig. 2) Hence, methanolic extracts of leaf, fruit, stem Fagonia and root of schweinfurthii at 366nm separates maximum number of components.

Table 1. Preliminary observation of Fagonia schweinfurthii Hadidi

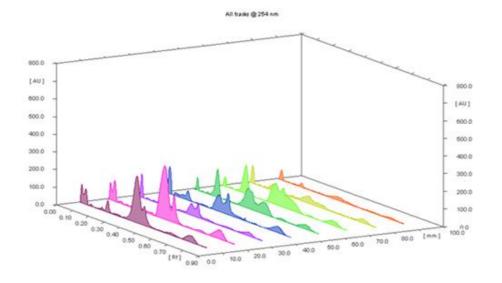
Plant parts	Alkaloids (Wagner's test)	Flavonoids	Steroids
Leaf	+	+	+
Stem	+	+	+
Fruit	+	+	+
Root	+	+	+

(+) = present;(-)= absent

Table 2. Rf values of *Fagonia schweinfurthii* Hadidi leaf, stem, fruit and root extract in Volume 20µl at 254 nm

Volume	Plant part Extracts	No. of peaks detected	Peak	Max Rf	Assigned Substance
	Leaf	8	1	0.01	Unknown*
20µl			2	0.04	Unknown*
			3	0.08	Unknown*
			4	0.18	Unknown*
			5	0.36	Unknown*
			6	0.42	Unknown*
			7	0.58	Unknown*
			8	0.71	Unknown*
	Stem	8	1	0.03	Unknown *
			2	0.07	Unknown *
			3	0.11	Unknown *
			4	0.15	Unknown *
			5	0.34	Unknown *
			6	0.40	Unknown *
			7	0.51	Unknown *
			8	0.72	Unknown *
	Fruit	7	1	0.03	Unknown *
			2	0.09	Autogenerated 1
			3	0.16	Unknown *
			4	0.35	Unknown *
			5	0.45	Unknown *
			6	0.56	Unknown *
			7	0.72	Unknown *
	Root	4	1	0.03	Unknown *
			2	0.15	Unknown *
			3	0.38	Unknown *
			4	0.40	Unknown *

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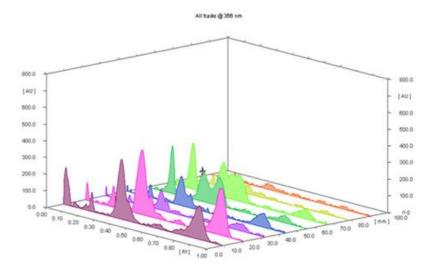


Fig. 1.

Fig. 2.

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Volume	Plant part Extracts	No. of peaks detected	Peak	Max Rf	Assigned Substance
20µl	Leaf	10	1	0.02	Autogenerated 4
			2	0.05	Autogenerated 6
			3	0.08	Autogenerated 9
			4	0.12	Autogenerated 10
			5	0.18	Autogenerated 7
			6	0.36	Autogenerated 1
			7	0.42	Autogenerated 2
			8	0.47	Autogenerated 3
			9	0.59	Autogenerated 25
			10	0.86	Unknown*
	Stem	12	1	0.03	Autogenerated 4
			2	0.07	Autogenerated 9
			3	0.15	Autogenerated 13
			4	0.25	Autogenerated 10
			5	0.34	Autogenerated 15
			6	0.40	Autogenerated 1
			7	0.48	Autogenerated 19
			8	0.51	Autogenerated 3
			9	0.46	Autogenerated 16
			10	0.64	Autogenerated 33
			11	0.67	Autogenerated 32
			12	0.86	Unknown*
	Fruit	13	1	0.01	Autogenerated 4
			2	0.06	Autogenerated 6
			3	0.16	Autogenerated 10
			4	0.35	Autogenerated 1
			5	0.41	Autogenerated 19
			6	0.44	Autogenerated 3
			7	0.61	Autogenerated 25
			8	0.64	Autogenerated 33
			9	0.72	Autogenerated 12
			10	0.80	Unknown*
			10	0.83	Unknown*
			12	0.86	Unknown*
			13	0.98	Unknown*
	Root		10	0.16	Autogenerated 10
	NOOL	12	2	0.23	Autogenerated 7
		12	3	0.25	Autogenerated 15
			4	0.20	Autogenerated 1
			4 5	0.38	Autogenerated 17
			5 6	0.38	-
			6 7	0.52 0.55	Autogenerated 16
			8	0.55	Autogenerated 30
			8 9		Autogenerated 32
				0.72	Autogenerated 12
			10	0.83	Unknown*
			11	0.85	Unknown*
			12	0.89	Unknown*

Table 3. Rf values of Fagonia schweinfurthii Hadidi leaf, stem, fruit and root extract in Volume 20μ l at 366 nm

4. DISCUSSION

The present study of phytochemical analysis of plant parts of Fagonia schweinfurthii Hadidi reveals the presence of secondary metabolites in each plant parts. Very few research work was done on these species. Pareek and Nagori (2013) studied antioxidative profiling of different extracts of Fagonia schweinfurthii Hadidi was done in which the total phenolics content from methanol extract of Fagonia schweinfurthii revealed 94.61 mg GAE/ g extract and Total flavonoids content from methanol extract Fagonia schweinfurthii revealed 21 mg QE/ g extract. From the methanol extract of Fagonia schweinfurthii maximum phenols and flavonoids were obtained in compared to petroleum ether extract, chloroform extract and ethyl acetate extract. For that's reason methanoilc extracts were taken for HPTLC. Avurvedic Pharmacopoeia of India volume five reported the Thin Layer Chromatography of the whole plant of Fagonia cretica Linn. was performed by using the mobile phase of chloroform: methanol: acetic acid in the ratio of 70:30:0.2,v/v/v under UV at 254 nm which elutes four spots, form those spots three spots were appeared in violet colored, their Rf values are 0.14, 0.32, 0.46 and a vellowish green colored spot at Rf 0.72. Under 366nm six fluorescent spots appeared. Brown colour spots were observed at Rf. 0.14, 0.32 while pink colored spots were observed at Rf 0.39, 0.51, 0.61 and 0.72. On exposure to iodine vapour eight spots were appeared at Rf. 0.14, 0.19, 0.28, 0.35 0.51, 0.61 and 0.72 all were in vellow color; at Rf 0.46 faint orange colored was observed. On spraying with vanillin sulphuric acid reagent and heating the plate at 110°C for 10 min. ten spots were appeared as follows [10].

Sr. no.	Rf value	Color
1	0.06	Bluish grey
2	0.14	Violet
3	0.19	Brown
4	0.2	Violet
5	0.35	Brown
6	0.39	Violet
7	0.46	Brown
8	0.51	Violet
9	0.61	Brown
10	0.72	Violet

Babu and Radhaswamy separated compounds from the ethanolic extract of leaf, stem and root parts of *G. pentaphylla*. The chromatograms under UV 254nm 9 peaks were separated from the leaf extract indicating the occurrence of 9 different components. The stem and root showed 4 and 5 peaks, respectively [11]. From the HPTLC analysis of plant parts of *Fagonia schweinfurthii* at 254 nm all peaks were detected are unknown except one and at 366nm all the peaks which are detected from the leaf and stem methanolic extracts are known except one. In leaf 10th numbered peak and in stem 12th numbered peak at Rf value 0.86 is unknown component, while in fruit 4 peaks at Rf values 0.80, 0.83, 0.86 and 0.98 are unknown and in root extract 3 peaks at Rf values 0.83, 0.85 and 0.89 are unknown.

Gupta et al [12] isolated 13 main secondary namely alkaloids, anthracene compounds derivatives, arbutin derivatives, bitter cardiac alvcosides. compounds. coumarin derivatives, essential oils, flavonoids, lignans, pungent-tasting principles, saponins, triterpenes and valepotriates from the bark, leaves and seeds of Careva arborea. Bark extracts showed the presence of 1alkaloid, 3 anthracene derivatives. 1 arbutin derivative, 7 bitter compounds, 3 cardiac glycosides, 2 coumarin derivatives, 7 essential oils, 5 flavonoids, 5 lignans, 4 pungent-tasting principles, 4 saponins, 4 triterpenes and 6 valepotriates. Leaf extracts showed the presence of 6 anthracene derivatives, 1 arbutin derivative, 8 bitter compounds, 8 cardiac glycosides, 4 coumarin derivatives, 7 essential oils, 9 flavonoids, 7 lignans, 6 pungent-tasting principles, 5 saponins, triterpenes and 6 valepotriates. Seeds 7 showed the presence of 1 anthracene derivative, 6 bitter compounds, 6 cardiac glycosides, 3 coumarin derivatives, 4 essential oils, 3 flavonoids. 6 lignans, 5 pungent-tasting principles, 5 saponins, 5 triterpenes, and 6 valepotriates [13].

5. CONCLUSION

Fagonia schweinfurthii (Hadidi) belongs to family Zygophyllaceae. There are no reports on phytochemical analysis of Fagonia schweinfurthii. Author investigated and collected Fagonia schweinfurthii from Bet Dwarka and Dwarka, Sea Coast of Gujarat. Preliminary screening method of phyto-constitute was followed by Sofowara1982; Trease & Evans, 1998. 1989 and Harbone, Preliminary investigation reveals the presence of alkaloids, flavonoids and steroids in the aqueous extract of plant parts. Phytochemical screening along with these HPTLC profiles is thus needed in setting up the standard of this plant. The present study provides evidence that plant parts of *Fagonia schweinfurthii* (Hadidi) methanol extracts contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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