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Phytochemical Screening and HPTLC Fingerprint Analysis of root extract of *Fagonia bruguieri* DC.

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ABSTRACT: Ayurvedic medicine is the ancient form of health care known to human being and has been used in all cultures throughout history. The validation of these natural products requires well equipped analytical devices and established methods of purification and utility. Keeping this in mind the present study was selected for preliminary phytochemical screening of the methanolic root extract of *Fagonia bruguieri* DC. Phytochemical studies showed the presence of chemical constituents like alkaloids, glycosides, Saponins, flavonoids and proteins. HPTLC fingerprint analysis of the root extract was carried out using CAMAG HPTLC system equipped with Linomat 5 sample applicator, UV Cabinet and WIN-CATS-4 software. The results from HPTLC fingerprint analysis of the methanolic root extract of *Fagonia bruguieri* revealed the presence of β -Sitosterol as chemical constituent. It can be concluded that HPTLC fingerprint analysis of root extract of *Fagonia bruguieri* can be used as a diagnostic tool for correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant population.

KEYWORDS: *Fagonia bruguieri*, phytochemical screening, HPTLC fingerprint.

I. INTRODUCTION

In Ayurveda since ancient times human ailments and diseases are treated with herbal medicine containing various chemical compounds. Depending on the ailment disease single or many herbs are mixed and administered to the patient in various forms viz. powder, extract in water, alcohol etc and their fermentation products. In order to meet their basic needs, man has always had a close relationship with plants (Rayn Clarenc Aarland et al., 2015). Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Mutha, R.E. et al., 2013). More than 422000 species of flowering plants have been reported from all over the world which 5000 species among them are used for medicinal purposes (Mozaffarian, V. 2005). Phytochemicals are biologically active, naturally occurring chemical compounds found in different parts of the plants, which provide health benefits for humans (Hasler, C.M. and Blumberg, J.B., 1999). There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga Rao, 2003).

Various extraction methods and analytical methods as spectrophotometry, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and Fluorescence Transmission-Infrared Spectroscopy (FT-IR) are developed for the determination of about plant active compounds (Aarland, R. et al, 2014). High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis.

HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug (Ram Mauji, 2011). HPTLC also facilitates repeated detection of chromatogram with same or different parameters (Karuppusamy Arunachalam et al., 2013 and Sushma, G.S., 2013). The present research deals with the phytochemical investigation and development of HPTLC fingerprints of ethanol extract of *Fagonia bruguieri* DC.

Fagonia bruguieri (family Zygophyllaceae) in vernacular it is known as 'Dhamasa'. The plant is small spiny under shrub, prostrate to sub erect. The stem is branched, stiff; braches are quadrangular, sulcate, striate, glandular and pubescent. Leaves are opposite 1 & 3 foliate, glandular and hairy. Leaflets are 0.4- 1.5cm long, linear, ovate to oblong sometimes slightly falcate, fleshy, long and mucronate. Stipular spines found near the leaves are straight, rarely slightly curved glandular and pubescent. Flowers are small 1.1cm across, pale pink, fading white, solitary and axillary with glandular hairy sepals. Fruit is capsule 4x3mm, pyramidal, pubescent, separating into five seeded cocci when dry, tipped with persistant style. Flowers and fruits are found during February-May (Singh et al., 2001).

This plant is found growing as weed in agricultural fields and it has number of medicinal properties. The plant is used as a bitter astringent tonic and febrifuge. It is employed in the preparation of Kumari Asava, an indigenous medicinal preparation known for its stimulative, laxative and alterative properties. It removes thirst fever and cures typhoid, asthma, tumours and delirium. The plant has antiseptic properties and is applied in the form of paste or poultice to abscesses, wounds and scrofulous glands. It is also given as a prophylactic against small pox. (Nadkarni, 2002) The twigs are used as a toothbrush and bark in scabies. (Kirtikar and Basu, 1990; Chopra et al. 1956). It is also used in cough, fever, dysentery and skin diseases. Boiled plant residues used for abortion. EtOH (50%) extract of aerial parts acts as antiviral, antiamphetamine and spasmog. Plant ash is given to children suffering from anaemia. Leaves and twigs decoction acts as blood purifier and given for skin lesions (boils and abscesses); claimed to cure tumour and cancer in preliminary stages. Stem, leaves and fruit powder shows an anti microbial property.

II. RELATED WORK

Phytochemical screening of *Fagonia bruguieri* revealed a variation in the presence of carbohydrates and / or glycosides, saponins, tannins, unsaturated sterols and/or triterpenoids, alkaloids, cardiac glycosides, cyanogenic glycosides, flavonoids, coumarins, chlorides and sulphates (Eman, A. Alam, 2010).

Allelopathic potential of *Fagonia bruguieri* was studied with respect to phytoextracts bioassay, leachates bioassay, volatilization bioassay, decomposition bioassay and root zone soil bioassay. The results obtained from bioassay are as follows.

a) Phytoextracts Bioassay: Aqueous extracts of root and leaf proved more inhibitory effects on hypocotyls elongation than radicle of *Cucumis* seedling. Root extract at 20 % w/v & 25 % w/v concentrations were more potent than leaf and stem extracts. The order of inhibition of phytoextracts of *Fagonia* was root > leaf > stem.

b) Leachates Bioassay: The root and stem leachates fully suppressed seed germination and seedling growth of test crop.

c) Decomposition (Decaying plant parts): The plant material at the rate of 32g / 250g of the soil caused maximum inhibition over their control. The inhibition in germination was inversely related to amount of plant material incorporated in the soil.

d) Volatilization: Water kept with plant material of *Fagonia bruguieri* in air tight jar showed maximum inhibitory effect on seed germination and seedling growth of test crop.

e) Root zone soil (Root exudation) Bioassay: During root zone soil bioassay seed germination as well as seedling growth of test crop was significantly inhibited over control.

f) Root zone soil analysis: Results of root zone soil analysis beneath *Fagonia bruguieri* showed less content of Organic Carbon. Available NPK was also found reduced amount.

From the above results obtained during laboratory bioassay clearly indicated that *Fagonia bruguieri* may releases some toxic chemicals through root phytoextract, root leachates, root exudation and causes the allelopathic effects. However, population dynamic studies corroborate with the bioassay results and hence, due to one or other reasons of it might show maximum dominance in the ecosystem. HPTLC finger print studies on root extract of *Fagonia bruguieri* has not carried out so far. Therefore, this work was taken for analysis and confirmation of β - Sitosterol as a major chemical constituent present in the roots of this medicinal plant.



III. MATERIAL AND METHODS

Mature and healthy plants of *Fagonia bruguieri* were collected from South east part of Pune District of Maharashtra State, India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the 'The Flora of the Presidency of Bombay Vol II by T. Cooke (Cook T.1958). Voucher specimens are documented in the herbarium of department of Botany, VPASC College, Baramati, Dist-Pune (MS), India.

Preliminary Phytochemical Screening:

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, Saponins, flavonoids etc. Preliminary phytochemical tests were carried out for the confirmation of starch, proteins, tannins, saponins, reducing sugars and anthroquinones on water extractives, while alkaloids, glycosides and flavonoids in alcoholic extractives with standard protocols (Johansen, D. A., 1940 and Krishnmurty, K. V., 1988). The preliminary phytochemical screening is a qualitative chemical evaluation which indicates spectrum of chemical constituents in the chosen plant. The chemicals and solvents used throughout the investigation were of analytical grade. The results were presented in Table 1.

HPTLC Studies (High Performance Thin Layer Chromatography):

HPTLC analysis on methanolic extract of *Fagonia bruguieri* was carried out by using standard protocol (Passera, et al., 1964).

HPTLC methodology:

One gram of extract of each added with 8 ml of methanol. Boiled for three minutes and made up to 15 ml in graduated tube. The solutions were filtered and 5 μ l of each extract was applied on Merck aluminum plate 10cmx10cm size precoated with silica gel of 0.3 mm thickness and for aristolochic acid the plate was developed in Toluene: Ethyl Acetate : Formic acid (10:3:05). For comparison 5 μ l standard β -Sitosterol was applied on Merck aluminium plate precoated with Silica gel. 10% Methanolic Sulphuric Acid was used as Derivatization Reagent. The plates were air dried and scanned at UV 254 nm and 366nm using deuterium lamp in CAMAG HPTLC instrument provided with win cats (Passera, et al., 1964). The qualitative results obtained by adding together the areas of every peak in the chromatogram were recorded. The results were depleted in the form of series of peaks in chromatogram each one representing a compounds in the mixture passing through the detector and absorbing UV light.

IV. RESULTS AND DISCUSSION

Preliminary Phytochemical Screening:

The vegetative part powders with various extracts were subjected to preliminary phytochemical screening and presented in Table 1. The vegetative parts powder with various extracts showed positives for phytochemicals like alkaloids, starch, tannins, proteins, Saponins and Flavanoids. Flavanoids therapeutically exhibits activities like analgesic, carminatives, anthelmintics, antiseptic, counter irritant.

Test	Root	Stem	Leaf
A) Water Extracts			
Starch	+ve	-ve	-ve
Proteins	+ve	-ve	+ve
Tannins	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Anthroquinones	-ve	-ve	-ve
Reducing Sugars	-ve	-ve	-ve
B) Alcohol Extracts			
Flavanoids	-ve	-ve	+ve
Alkaloids	+ve	+ve	+ve
Dragendorff's Reagent	+ve	+ve	+ve
Mayer's Reagent	-ve	-ve	-ve
Wagner's Reagent	-ve	-ve	-ve

Table 1: Results showing Preliminary Phytochemical Screening of *Fagonia bruguieri* DC.



Fig. 1. *Fagonia bruguieri* DC. Growing in Agricultural Filed



Fig. 2. Flowering twig of *Fagonia bruguieri* DC.



Fig.3. Root, Leaves and fruits of *Fagonia bruguieri*

HPTLC studies:

The results from HPTLC fingerprint scanned at different wavelengths for methanol and aqueous extract of *Fagonia bruguieri* root confirmed the presence of β -Sitosterol as secondary metabolites with different concentrations. It can be concluded that HPTLC fingerprint analysis of root extract of *Fagonia bruguieri* can be used as a diagnostic tool for correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant population. The HPTLC finger print scanned at wavelengths 254 nm and 366nm for methanolic extracts of *Fagonia bruguieri* was obtained as shown in the Figures 3.



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