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PHARMACOGNOSTICAL EVALUATION AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *JUSTICIA ADHATODA* LEAVES EXTRACT

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ABSTRACT

Natural products have been implemented as alternative health care treatment and in discovery of modern drugs. A major focus of natural product chemistry has been toward drug design and discovery. *Justicia adhatoda* is a well-known Indian medicinal plant valued for its pharmacopeia. This plant root, bark, leave and flower are used to heal many types of infection. The main objective of the present work was designed to evaluate the pharmacognostic characters and qualitative phytochemical screening of *J.adhatoda* leaves extracts. The extracts were subjected to qualitative phytochemical screening using standard procedures. Result shows that many of the phytochemicals were present. They are Alkaloids, Flavonoids, Glycosides, Cardiac glycosides, Coumarins, Hydroxy anthraquinones, Tannins, Phlobatannins, Proteins, Xantho protein, Steroids and Phenols. However Amino acids and Trepenoids were found absent in leaf extract. The diversity of phytochemicals found present suggests that *J.adhatoda* leaf could serve as a source of useful drug.

KEYWORDS: *Justicia adhatoda* leaves, pharmacognostic study, phytochemicals, ash value, drug.

1. INTRODUCTION

Medicinal plants represent a rich source of antimicrobial and phytochemical agents. Plants are used medicinally in different countries of world and as source of many powerful drugs. A wide range of medicinal plant parts is used for extraction of raw drugs as they possess varied medicinal properties [1]. Plants are believed to be important source of new phytochemical with potential therapeutic effects. Traditional medicinal plants should be able to play a greater role in the modern primary oral healthcare system of many countries. Many studies have been reported that the plants are as good as the conventional ones [2-3]. In world different metabolites were known. Few of them were used in our daily life such as the flavonoids, proteins, lycopene [4]. Plants produce the active composites that were used for curing different ailments of people. The phytochemical examination provides vast awareness to pharmacist and helps them in creating diverse medicines to cure different ailments [5]. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently envogue in parts of the world. Dulsa (*Justicia adhatoda*) is a small evergreen herbal plant in the family *Acanthaceae*. It is distributed all over the plains of India and in lower Himalayan ranges.

Vasicine produced by *Justicia adhatoda* is used for the treatment of various diseases and disorders, mostly for respiratory tract ailments. Present study is carried out in *J.adhatoda* leaves to pharmacognostical evaluation and screening of its phytochemical profile.

2. MATERIALS AND METHODS

2.1 Sample collection

The fresh leaves of *Justicia adhatoda* was collected from Kurumbalur village, Perambalur district, Tamilnadu state, India. The collected plant material, *i.e. J.adhatoda* leaves, were shade dried for about a week and grinded in the form of powder.

2.2 Preparation of plant extract

The dried plant leaves were powdered and extracted (25g) exclusively with 100 ml each of petroleum ether, chloroform, acetone, ethanol, diethyl ether and water in a soxhlet extractor for 4 hrs. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°C). All the extracts were preserved in a refrigerator until further use.[6]

2.3 Phytochemical analysis

The phytochemical analysis of *Justicia adhatoda* leaves was carried out by the standard method that previously described.[7-8]

2.4 Fluorescence analysis test

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in various radiations were recorded.[9-10]

3. RESULTS AND DISCUSSION

The present study was carried out on the plant *J.adhatoda* leaves to reveal the presence of medicinally active constituents and its pharmacognostical properties. Pharmacognostical and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug samples.[11] Current investigation was considered to estimate and confirm the presence of phytochemicals in different extracts of *J.adhatoda*.

3.1 Physicochemical characteristics

The dried and powdered leaf was green in colour and has a bitter taste in nature. The leaf powder has a strong odor. Physicochemical parameters like loss on drying, ash values, acid insoluble ash and extractive values are given in Table 1.

Table 1: Loss on drying and ash values of *Justicia adhatoda* leaf powder.

S. No	Parameters	Values of three replicates (% w/w)	Mean ± SEM
1	Loss on drying (LOD)	11.4	11.43 ± 0.43
		10.3	
		12.6	
2	Ash values: 1. Total ash	5.32	5.31 ± 0.47
		5.73	
		4.89	
3	2. Acid soluble ash	1.98	1.95 ± 0.62
		2.02	
		1.87	
4	3. Water soluble ash	2.85	2.56 ± 0.23
		2.48	
		2.37	

Values are expressed as Mean ± Standard Error ($P<0.001$).

3.2 Results of phytochemical analysis of *J.adhatoda* leave extracts

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.[12]

The qualitative phytochemical analysis of different extracts of *J.adhatoda* leaves was showed in Table 2. The phytochemical analysis results revealed that the presence of alkaloids, flavonoids, glycosides, tannins, proteins, phenols and resins.

Table 2: Preliminary phytochemical screening of extract of powdered leaves of *Justicia adhatoda*.

S.No	Name of the Compounds	Name of the polar solvents			Name of the non polar solvents		
		Acetone	Ethanol	Water	Chloroform	Diethyl ether	Petroleum ether
1	Alkaloids	++	-	-	++	++	+
2	Flavonoids	+++	-	++	+	+	-
3	Carbohydrates	-	-	+	-	-	-
4	Glycosides	+++	+	+++	-	-	+
5	Cardiac glycosides	+	-	+	-	-	-
6	Coumarins	+	+	-	+	-	-
7	Saponins	-	+	+	+	-	-
8	Hydroxyanthraquinones	+	-	+	-	-	-
9	Tannins	+	+	+	+	+	-
10	Phlobatannins	+	-	+	-	-	-
11	Proteins	++	++	++	++	+	-
12	Xantho protein	+	-	+	+	+	-
13	Amino acids	-	-	-	-	-	-
14	Steroids	+	-	-	-	-	-
15	Terpenoids	-	-	-	-	-	-
16	Phenols	+	+	+	++	+	+
17	Resins	-	+	+	+	+	+
18	Volatile oil	+	-	-	+	-	-
19	Fatty acid	+	-	-	-	-	+
20	Emodins	+	-	-	-	+	-

Note: + → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; -- → absent.

Based on this results acetone, chloroform and water extracts was showed the presence of different types of phytochemicals when compared to the other extracts. When compared to these three extracts, acetone having the very good phytochemical profile.

The presence of alkaloids was observed in acetone, chloroform and water extract in medium concentration and petroleum ether extract in small concentration. The flavonoids were detected in very high concentration on acetone extract and medium concentration in water extract. The glycosides were present in very high concentration on acetone and water extract. The saponins were present in small concentration in ethanol, water and chloroform extracts. The presence of tannins was observed on acetone, ethanol, water, chloroform and diethyl ether extract in very small concentration. The protein was present in medium concentration in acetone, ethanol, water and chloroform extracts. The phenols and resins were present in all the extract of *J.adhatoda* leaves.

Tannins were strong bioactive compounds found in medicinal plant often meet in food artifacts of plant parts that can be used for beneficial reason. Flavanoids have antioxidant properties and used in case of irritation, against microorganisms.[13] Glycosides were used as stimulant in case of cardiac collapse.[14] Sterols that were present in different parts of *D.bupleuriodes* have been stated to used as antibacterial activities important compound as sex hormones.[15] Saponin that was present in leaves and stem was used to prevent blood losing and in curing injuries.[16]

These composites provided accepted defense as antibiotics, which facilitate the body to struggle against diseases and microbial attack.[17]

3.3 Fluorescence analysis

The ultra violet light produces fluorescence in several natural products, which do not noticeably fluoresce in daylight. If the substances themselves are not fluorescent, they may often be rehabilitated into fluorescent derivatives or decomposition products by applying diverse reagents. Therefore, some crude drugs are often assessed qualitatively in this technique and it is an imperative parameter of pharmacological evaluation.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products (e.g. alkaloids like berberine) which do not visibly fluoresce in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation.[18-19]

Fluorescence analysis of leaf powder was carried out after treating with several solvents. Fluorescence was observed at 254 and 365 nm comparing its change of colour in visible light.

The observations are presented in Table 3 and Table 4 showing the variation in colour.

Table 3: Fluorescence analysis of powdered leaves of *Justicia adhatoda.*

S.no	Chemical Treatment	Day light	UV Light	
			254 nm	365 nm
1.	Powder +1M H_2SO_4	Pale greenish yellow	Violet	Violet
2.	Powder +10% $CuSO_4$	Green	Purple	Purple
3.	Powder +1M HCl	Brown	Violet	Violet
4.	Powder +Diluted HNO_3	Brown	Violet	Violet
5.	Powder +10% NaOH	Green	Green	Green
6.	Powder +1% glacial acetic acid	Green	Pale Green	Green
7.	Powder +Concentrated HNO_3	Brown	Green	Green
8.	Powder+ Concentrated HNO_3+ Diluted HNO_3	Pale Yellow	Pale Yellow	Pale Yellow
9.	Powder+ 1% Iodine	Pale Yellow	Purple	Purple
10.	Powder+ Ethanol	Green	Pink	Pink

Table 4: Fluorescence analysis of leaves extract of *Justicia adhatoda.*

S.no	Chemical Treatment	Day light	UV Light	
			254 nm	365 nm
1.	Extract +50% aqueous.NaOH	Green	Black	Black
2.	Extract +50% alcohol.NaOH	Green	Blackish Pink	Blackish Pink
3.	Extract +Concentrated HCl	Green	Black	Black
4.	Extract +50% HCl	Green	Pink	Pink
5.	Extract+50% HNO_3	Blackish Green	Blackish Purple	Blackish Purple
6.	Extract +Concentrated HNO_3	Blackish Red	Pinkish Black	Pinkish Black
7.	Extract +50% H_2SO_4	Blackish Green	Pinkish Brown	Pinkish Brown
8.	Extract +Concentrated H_2SO_4	Blackish Green	Blackish Pink	Blackish Pink
9.	Extract +Methanol	Green	Pinkish Black	Pinkish Black
10.	Extract +Ammonia	Green	Black	Black
11.	Extract +1% Iodine	Green	Brownish Red	Brown
12.	Extract+ 10% $Fecl_2$	Green	Brown	Brown
13.	Extract+ Concentrated glacial acetic acid	Blackish Green	Pinkish Black	Pinkish Black

Based on the above findings it was finally evaluated that this plant was very important medically because of their presence of wide variety of phytochemicals. Therefore this study recommended advance study of this plant. The extract needs further investigation for the mechanism of action and potential to be employed in various human ailments.

4. CONCLUSION

It has been identified that pharmacological studies and phytochemical studies were revealed that this plant have a wide variety of medically important phytochemicals. The further research in this plant may help in determining the therapeutic potential of *Justicia adhatoda*.

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