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Preliminary phytochemical screening of *Salvadora oleoides* Decne (Salvadoraceae)

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Abstract

The plant *Salvadora oleoides* Decne is a small herb, grows particularly well along rocks and in the dry mountainous areas of Gujarat. The leaves and flowers are used as cooling agents and as blood purifiers. The leaf paste is applied on an open wound and inflamed areas by the local people of this region. The pharmacognostical and phytochemical studies of leaf of this plant have not been reported. Therefore, the present investigation was planned to study the pharmacognostical and phytochemical aspects of Salvadora oleoides. Morphological and microscopical examination of *Salvadora oleoides* leaf was carried out using the reported methods in standard texts. Physical constants of crude drugs like loss on drying, ash values and extractive values were determined. Leaf constants like stomatal number, stomatal index, pallisade ratio, vein islet numbers and vein termination numbers were also determined to establish the standards. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilage were present in the leaf extracts of *Salvadora oleoides*. The thin layer chromatography was also carried out as per the standard texts.

Key-Words: *Salvadora oleoides*, Physical constants, Leaf constants, Preliminary phytochemical screening and TLC profile

Introduction

Salvadora oleoides Decne. (Salvadoraceae) grows naturally by seed germination and is one of the dominant tree species in the vast area of Kutch (northern saline desert). It also grows successfully in the coastal area as well as in the non-saline and marginal semi-arid (closer to arid) central area. This tree species is of multipurpose use because of its oil-yielding potential, pharmaceutical application, fodder and fuel values and many others. The plant possesses good medicinal value and is used by the people for the treatment of various diseases. Decoction of leaves was given to the cattles to promote the expulsion of dead fetus from the uterus. The leaves and flowers were used as a cooling agent and blood purifier. The leaf paste was applied on an open wound and also useful in inflammation of legs. Stem possesses an anthelmintic and diuretic activity. Root bark is used in the treatment of piles. Seeds are used in the treatment of cough. The whole plant is used as diuretic, cooling herb, anti-inflammatory agent, wound healing herb and nerve tonic[1].

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Only two species of *Salvadora* is found in Gujarat i.e. *Salvadora oleoides* and *Salvadora phlomoides*. It is a small herb, grows particularly along rocks and in the dry mountainous areas of Gujarat. The plant generally grows during rainy season (June – September). The whole plant is traditionally used in the treatment of various uterine and skin disorders by the local people of Kachchh region[1-2].

The main objectives of the present research work are to establish its botanical standards which are helpful for the future identification and authentification of the plant and to know the pharmacologically active constituents present in the plant.

Material and Methods

Collection and Identification of Plant

Leaves of *Salvadora oleoides* Decne were freshly collected from the Kachchh district of Gujarat (India) in the month of July. The plant material was identified and authenticated by the Botany Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat (India). The voucher specimen SOPMA-01 was also preserved for future reference. The collected leaves were shade dried for 15 days and

size reduced by mechanical grinder into coarse powder. It was then stored in a well closed container free from environmental climatic changes till usage.

Morphological and microscopical investigations[3-8]

Morphological examination of *Salvadora oleoides* leaf was carried out using the reported methods in standard texts. Microscopical characters were studied by preparing the specimen leaves of *Cordia rothii* in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of TBA (tertiary-Butyl alcohol) as per the schedule. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60 ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. Wherever necessary, sections were also stained with safranin, fast-green and N/10 iodine to identify the presence of lignified cells and starch grains.

Physical constants[9-10]

Physical constants of crude drugs like loss on drying, ash values and extractive values were determined by using official methods. Following determinations were made:

Leaf constants[9-10]

Leaf constants like stomatal number, stomatal index, pallisade ratio, vein islet numbers and vein termination numbers were also determined to establish the standards.

Extraction of plant material[11-12]

The powder of dried leaves of *Salvadora oleoides* was subjected to continuous extraction with soxhlet extractor using various organic solvents such as petroleum ether (60-80 °C), chloroform, ethyl acetate, ethanol and water respectively. After concentration and drying of each extract, identification of phytoconstituents was carried out by performing different qualitative chemical tests. The colour, consistency and percentage yield of the extracts were also noted.

Preliminary phytochemical screening of various extracts[11-14]

The leaf extracts of *Salvadora oleoides* obtained during the extraction process were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituentsy using reported methods. Following chemical tests were performed:

Thin layer chromatography of various extracts[12-13]

After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out by thin layer chromatography using different detecting reagents. The test extract was dissolved by using appropriate solvent in a concentration of 1 mg/ml and subjected for spotting. Silica gel G (mesh size 60) was used as a stationary phase and appropriate solvent systems to determine the presence of various phytoconstituents. The R_f value of compounds were noted for all the extracts.

Results and Discussion

Morphological investigations

The pharmacognostic parameters are helpful for the future identification and authentification of the plant in the herbal industry. The leaf constants can be included as microscopical standards in Indian Herbal Pharmacopoeia.

Morphological examination of leaf reveals that the plant *Salvadora oleoides* is a shrub or tree; branches stiff, rough, whitish. Leaves coriaceous; petiole 2-l.2 cm long; lamina 1.5-7.5 cm long, 4-1.5 cm broad, elliptic-lanceolate, mostly acute, rarely obtuse or mucronate glabrous with obscure lateral veins. Inflorescence axillary panicles, or branched spikes, 2.5-4 cm long. Flowers greenish white, 2-3 mm across; pedicel 1 mm long or absent. Calyx 1.5-2 mm long, with round lobes and wavy margin, divided nearly half way down, glabrous. Corolla 2.5 mm long, obovate or oblong; lobes sub-acute and recurved. Stamens 4, inserted at the base of the corolla tube. Style absent, stigma peltate. Fruit a drupe, 5 mm in diameter, globose, yellow on maturation. Seeds greenish-yellow, about 3 mm in diameter.

Microscopical investigation

Leaves: Both surfaces with stomata, sunken and almost the same size and uniformly distributed on both surfaces. This specie has very distinct pattern on both the surface of leaf as compared to other species of *Salvadora*. Epidermal cells are of two different shapes, few are circular and bulging, and others are forming rows of elongated suppressed cells. Both surfaces of leaf show trichomes which are very sparsely distributed on the surface but found more near to the margin. Trichomes are simple non glandular and unicellular (approximate size 35.1-36.2µm). The presence of trichome on leaf surface is a distinguishing character of this species.

Branches: Branches spreading; stiff, yellow green, surface glaucous with trichome. The presence of trichomes on branch surface is a distinguishing character of this species.

Seeds: Globular, 6.54 µm long and 5.24 µm wide, brown in color. Seed surface show reticulate pattern with small pits. The cells are regularly or irregularly arranged in circular rows.

Physical constants

The physical standards, such as loss on drying, ash values, extractive values will be useful to identify the

authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in the future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species.

Physical evaluation revealed that loss on drying 6.05 %; total ash 5.34 %; acid insoluble ash 2.47 %; water soluble ash 6.18 %; alcohol soluble extractives 12.46% and water soluble extractives 17.22 % w/w values were observed in leaves of *Salvadora oleoides*. The results were shown in **Table 1**.

Leaf constants

Leaf constants are fixed for all plant species, but they may vary from species to species. Determination of leaf constants is also one of the methods of standardization. It is helpful in identification of correct plant variety and also useful in predicting adulteration. The results of leaf constants determined are tabulated as under **Table 2**.

Extractive values (%w/w)

During successive solvent extraction, the percentage yields were determined as petroleum ether 7.97 %, chloroform 4.34 %, ethyl acetate 4,78 %, ethanol 8.66 % and water 11.40 % w/w. The colour and consistency of the each extract were also noted during the extraction process as shown in **Table 3.**

TLC profile of extracts

The preliminary phytochemical studies with the help of thin layer chromatography revealed the presence of saponins in water extract (R_f value 0.44, 0.49, 0.56); phytosterols in chloroform and alcohol extract (R_f value 0.34, 0.37); triterpenes in chloroform extract (R_f value 0.69); carbohydrates in alcohol and water extract (R_f value 0.57, 0.59, 0.64); fats and oils in petroleum ether, chloroform, ethyl acetate and alcohol extract (R_f value 0.73, 0.79). The results were shown in **Table 4**.

Preliminary phytochemical screening of *Salvadora oleoides* leaves

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols *etc*. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates and glycosides, saponins, tannins, triterpenes, mucilage, fats and oils, were present in the leaf extracts of *Salvadora oleoides*. The results were shown in **Table 5**.

Conclusion

As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The pharmacognostic parameters are helpful for the future identification and authentification of the plant in the herbal industry. This study on micro-morphological features of *Salvadora oleoides*, proposed a set of anatomical parameters may enable those who handle this plant to maintain its quality control. Morphological as well as microscopical studies of plants are the primary steps to establish its botanical standards before going to other studies. The physical standards, such as loss on drying, ash values, extractive values will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in the future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. The leaf constants can be included as microscopical standards in Indian Herbal Pharmacopoeia. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols *etc*. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

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Fig. 1: Leaves of *Salvadora oleoides*

Table 1: Physical parameters of leaves of *Salvadora oleoides*

S/No.	Parameters	Determined value* (% w/w)
1.	**Loss on drying**	6.05 ± 0.28
2.	**Ash values**	
	Total ash	5.34 ± 0.21
	Acid insoluble ash	2.47 ± 0.03
	Water soluble ash	6.18 ± 0.05
3.	**Extractive values**	
	Alcohol soluble extractives	12.46 ± 0.63
	Water soluble extractives	17.22 ± 0.43

* Mean value of three counts

Table 2: Determination of leaf constants

S./No.	Parameters	Range	Mean
1.	Palisade ratio	6.00 – 8.00	6.64 ± 0.24
2.	Stomatal number-upper surface	7.00 – 9.00	7.98 ± 0.67
3.	Stomatal number-lower surface	5.00 – 8.00	6.34 ± 0.22
4.	Stomatal index-upper surface	20.42 – 23.22	21.08 ± 0.52
5.	Stomatal index-lower surface	12.62 – 14.16	13.36 ± 0.17
6.	Vein-islet number	3.00 – 4.00	3.48 ± 0.09
7.	Vein-termination number	2.00 – 3. 00	2.52 ± 0.12

Table 3: Percentage yield of leaf extracts of *Salvadora oleoides*

S./ No.	Solvent used	Colour and consistency	Average percentage yield (% w/w on dry weight basis)
1.	Petroleum ether (60-80° C)	Greenish sticky mass	7.97
2.	Chloroform	Brown sticky mass	4.34
3.	Ethyl acetate	Brown sticky mass	4.78
4.	Ethanol	Dark brown mass	8.66
5	Water	Dark brown mass	11.40

Table 4: Qualitative TLC analysis of phytoconstituents in *Salvadora oleoides*

Phytoconsti -tuents	Solvent system	Visualizing reagent	No. of Spots	R_f Value	Colour
Saponins	Ethyl acetate: Methanol (50:50)	Phenol sulphuric acid	3	0.44, 0.49, 0.56	Yellow-brown
Phytosterols	Pet.ether:Acetone (90:10)	Antimony trichloride	2	0.34, 0.37	Blue
Triterpenes	Chloroform : Acetone (80:20)	Libermann-Burchard reagent	1	0.69	Brown pink
Carbohydrates	Butanol:Glacial acetic acid:Ether:Water (45:30:15.5)	Phenol Sulphuric acid	3	0.57, 0.59, 0.64	Brown black
Fats and oils	Toluene:Ethyl acetate (93:7)	Vanillin sulphuric acid	2	0.73, 0.79	Yellow-brown

Table 5: Preliminary phytochemical screening of leaf extracts of *Salvadora oleoides*

	Plant constituents / Tests/reagents used	Extracts				
		P	**C**	**E**	**A**	**W**
1.	**Alkaloids**					
	Dragendorff's reagent	-	-	-	-	-
	Mayer's reagent	-	-	-	-	-
	Hager's reagent	-	-	-	-	-
	Wagner's reagent	-	-	-	-	-
2.	**Carbohydrates and glycosides**					
	Molisch's reagent	-	-	-	+	+
	Fehling solution	-	-	-	+	+
	Benedict's reagent	-	-	-	+	+
	Barfoed's test	-	-	-	-	-
	Baljet test	-	-	-	-	-
	Legal's test	-	-	-	-	-
	Borntrager's test	-	-	-	-	-
3.	**Phytosterols**					
	Salkowski test	-	+	-	+	-
	Liebermann-Burchard's test	-	+	-	+	-
4.	**Fixed oils and fats**					
	Spot test	+	+	+	+	-
	Saponification test	+	+	+	+	-
5.	**Saponins**					
	Foam test	-	-	-	-	+
6.	**Phenolic compounds**					
	Ferric chloride solution	-	-	-	-	-
	Bromine water	-	-	-	-	-
	Lead acetate solution	-	-	-	-	-
7.	**Tannins**	-	-	-	-	-
8.	**Triterpenes**	-	+	-	-	-
9.	**Flavonoids**	-	-	-	-	-
10.	**Proteins and aminoacids**					
	Millon's reagent	-	-	-	-	+
	Biuret test	-	-	-	-	+
	Ninhydrin reagent	-	-	-	-	+
11.	**Gums and mucilages**	-	-	-	-	+
12.	**Volatile oils**	-	-	-	-	-

[P - Petroleum ether extract; C - Chloroform extract; E – Ethyl acetate extract; A – Alcohol (Ethanol) extract; W - Water extract]