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Research Article

PHYTOCHEMICAL INVESTIGATION, SPECTRUM ANALYSIS OF *SALVADORA PERSICA* AND *CRESCENTIA CUJETE*

M.Dhanasekaran *, M.Udhayaeswaran and C.Rahul Dravid
PG and Research Department of Botany, The American College, Madurai - 2, Tamil Nadu, India
*Corresponding Author Email: m.dhana66@gmail.com

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ABSTRACT

Salvadora persica is a well-known medicinal plant which belongs to the family Salvadoraceae. It is commonly known as the Meswak tree. Calabash tree or *Crescentia cujete* tree belongs to the family of Bignoniaceae. It is also known as the gourd tree. These two plants have been studied by preliminary phytochemical and FTIR analysis. Data gathered on solvent extraction and preliminary phytochemical method suggested that the presence of primary and secondary metabolites in leaf tissue. Two solvents such as ethanol and aqueous are used here to reveal the phytocompounds and extend our work to find out the various functional groups present in these two plants through FTIR analysis was done. Spectrum of *Salvadora persica* showed 5 peaks that are 599.89, 654.86, 1409.06, 1431.24, 2930.96 and spectrum of *Crescentia cujete* showed that 13 peaks that are 470.65, 520.80, 630.75, 652.93, 776.38, 1060.89, 1155.41, 1248, 1321.30, 1431.24, 1527.69, 2860.56, 2924.21. The present study revealed that the functional groups of both plants, such as alcohols or phenols, alkanes, amines, esters or carboxylic acid or lactones, aldehyde or ketones, acetates and ethylene.

Keywords: *Crescentia cujete*, *Salvadora persica*, FTIR analysis, Functional groups, Phytochemical analysis.

INTRODUCTION

Salvadora persica is a well-known medicinal plant which belongs to the family Salvadoraceae. It is commonly known as the Meswak tree, because the roots and twigs of this tree have been used for oral hygiene and dental care. It is found in shrub savannah, from northwestern India to Africa. It is an evergreen shrub, 4- 6 m tall with a short trunk, white bark and smooth green leaves[1].

Salvadora persica of roots are used for treatment of gonorrhea and relieve the pain due to spleen troubles. Bark is used for treatment of fevers. Leaves are used for treatment of asthma, cough and piles. Carminative and diuretically properties of fruits are used for treatment of rheumatisms[2]. It has a capacity to treat these diseases due to the presence of biologically active compounds such as salvadoricine, salvadourea, trimethyl amine, di-benzyl thiourea, rutin, thioglucoside, chlorine, potash, sulphur etc[3]. Through traditional knowledge, it is believed that *Salvadora persica* has capacity to reduce blood cholesterol level. However, it is essential that before its repeated use for medicinal purpose, it should be subjected to botanical inspection to avoid side effects in the body. Since, phytosterols are added to foods because of their capacity to reduce the absorption of cholesterol in the gut and thereby it lowers the blood cholesterol level of the human body[4].

The seeds of *Salvadora persica* containing about 40% oil with a fatty acid composition (lauric-20%, myristic-55%, palmitic 20% and oleic-5%) which can make an excellent soap. Seeds also contain fluoride and silica[5]. Seed's bark and leaves are reported to have fatty acid methyl ester (FAME), tocopherol (γ-tocopherol, α-tocopherol, vitamin E, and γ-tocotrienols), sterol (phytosterol, sitosterol, β-sitosterol, campesterol), stearic acid and phenolic compound[6]. The plant contains sulphur[7], organic sulphur compounds, ascorbic acid[8, 9] and small amount of saponin. The chemical analysis of miswak sticks showed the presence of fluoride, calcium, phosphorus and silica. Fruits contain large amount of sugar, fat, colouring matter and an alkaloid, oilcake from the seeds contains nitrogen 4.8% potash 2.8% and phosphoric anhydride 1.05% ash contains large amount of chlorine[10].

Calabash tree or *Crescentia cujete* tree belongs to the family of Bignoniaceae. It is also known as the gourd tree. The calabash tree is 6 to 10 m tall with a wide crown and long branches covered with clusters of tripinnate leaves and gourd-like fruit. The branches have simple elliptical leaves clustered at the anode. The greenish flowers arise from the main trunk and blooms at night[11]. It is propagated either by seed or stem cuttings. Calabash fruit is a seasonal fruit that develops after pollination by bats. It appears at the end of dry season, and the fruit is up to 12 to 14 cm in diameter. It is globular with smooth hard green woody shell. It takes about six to seven months to ripen and eventually falls to the ground. Small flat seeds are embedded in the pulp[12]. The plant principally contains tartaric acid, cianhidric acid, citric acid, crescentic acid, tannins, beta- sitosterol, alpha and beta amyrins, stearic acid, palmitic acid, flavonoids (quercetin and apigenin), naphthoquinones, iridoid glycosides, 3-hydroxyoctanol glycosides[13].

Calabash is used in various parts of the world for their laxative, expectorant, anthelmintic, analgesic, anti-inflammatory and febrifuge properties. An antimicrobial property of the fruit leaves and bark of the Calabash tree has also been explored in various studies[14]and has yielded promising results. Of particular interest to this study is the fruit of *Crescentia cujete* or Calabash tree. Phytochemical study on the fruit yielded saponins, flavonoid, cardenolides, tannins, and phenol, as well as the presence of hydrogen cyanide.

Extracts from the various parts of *C. cujete* possessed anti-inflammatory[15], antibacterial[16], DPPH radical scavenging[17], antioxidant[18], CNS depressant[19], wound healing activities[20]. The present investigation aimed to screen out the phytochemicals from leaf extracts of *Salvadora persica* and *Crescentia cujete*

MATERIALS & METHODS

Plant materials

Salvadora persica

Salvadora persica is a small tree or shrub with a crooked trunk, typically 6–7 meters (20–23 ft) in height. Its bark is scabrous and cracked, whitish with pendulous extremities. The root bark of the tree is similar in colour to sand, and the inner surfaces are an even lighter shade of brown. It has a pleasant fragrance, of cress or mustard, as well as a warm and pungent taste. The leaves break with a fine crisp crackle when trodden on. The tree produces small red edible fruits, juicy but pungent, in clusters.

Crescentia cujete

Crescentia cujete is a smooth, much-branched tree growing to a height of 4 to 5 meters. Branches are arching with close-set clusters of leaves. Leaves are alternate, often fascicled at the nodes, oblanceolate, 5 to 17 centimeters long, glossy at the upper surface, blunt at the tip and narrowed at the base. Flowers develop from the buds that grow from the main trunk, yellowish, sometimes veined with purple, with a slightly foetid odor, occurring singly or in pairs at the leaf axils, stalked and about 6 centimeters long, and opens in the evening. Calyx is about 2 centimeters long and split into two lobes. Fruit is short stemmed, rounded, oval or oblong, green or purplish, 15 to 20 centimeters in diameter. Voucher specimen is preserved as herbarium in department of botany (ACH 2018,2019), The American College, Madurai.

Preparation of crude plant extracts

Solvent extraction

The collected plant materials (leaves of *Salvadora persica* and *Crescentia cujete*) were washed with running tap water to remove dusts and other particles. Then the materials were shade dried and blundered as coarse powder. The powder materials (200 gm) were subjected to extraction with solvent (ethanol) in Soxhlet's apparatus with continuous 8 hrs reflux and also. The crude extracts concentrated in room temperature and stored in desiccator.

Aqueous extract

The dust free plant material was ground with mortar and pestle in adequate amount of sterile distilled water. The homogenized tissue was standing out for separation (Cold maceration). The supernatant was taken as aqueous extract.

Qualitative phytochemical analysis

The concentrated extracts were used for preliminary phytochemical screening. The concentrated extracts are dissolved again in solvents (ethanol and aqueous) respectively and used for screening. The following tests were conducted for screening the phytocompounds[21].

Test for carbohydrates (Molisch's test)

2 ml of plant extract in a boiling tube added with 2 ml of Molisch's reagent and few drops of Conc. Sulphuric acid. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins (Ferric chloride test)

2 ml of plant extract, 2 ml of 5% ferric chloride is added in a boiling tube. The mixture turned into dark blue or greenish black indicates the presence of tannins.

Test for saponins (Foam test)

2 ml of plant extract and 2 ml of distilled water is added and shaken gently in a graduated cylinder for 15 minutes. Appearance of foaming layer indicates the presence of saponins.

Test for flavonoids

2 ml of plant extract added with NaOH (Sodium hydroxide) solution in a test tube. Appearance of yellow colour indicates the presence of flavonoids.

Test for alkaloids (Mayer's test)

2 ml of plant extract and 2 ml of concentrated hydrochloric acid is added in a test tube. Then few drops of Mayer's reagent (Potassium Iodide + Mercuric Chloride) are added. The appearance of green color or white precipitate indicates the presence of alkaloids.

Test for quinines

2 ml of extract and 2 ml of Concentrated Sulphuric acid is added. The solution turn into red color indicates presence of quinines.

Test for glycosides

2 ml of plant extract, 2 ml of chloroform and 2 ml of acetic acid gradually added alone the wall of test tube. The solution changed blue to green color that indicates presence of glycosides.

Test for Cardiac glycosides (Keller Kiliani test)

2 ml of extract, 2 ml of glacial acetic acid and few drops of 5% Ferric chloride added in a test tube. The mixture will be added 1 ml of concentrated Sulphuric acid on the top layer. The solution mixture changed into brown ring at the middle indicates presence of cardiac glycosides.

Test for Terpenoids

2 ml of extract, 2 ml of chloroform added, and concentrated Sulphuric acid added carefully in a test tube. Appearance of reddish-brown color at the junction of layer indicates presence of terpenoids.

Test for Phenols

2 ml of the extract, 2 ml of Ferric chloride solution added in a tube. Appearance of orange yellow colour indicates presence of phenols.

Test for coumarins

2 ml of extract, 2 ml of 10% sodium hydroxide added. Formation of yellow color indicates presence of coumarins.

Phytosteroids

2 ml of plant extract equal volume of chloroform is added in a test tube and then added few drops of concentrated Sulphuric acid. Appearance of bluish brown ring indicates the presence of phytosteroids.

Phlobatannins

2 ml of plant extract few drops of 2% Hydrochloric acid added in a test tube. The mixture changed into red color precipitate that indicates the presence of phlobatannins.

Anthraquinone

2 ml of plant extract and with few drops of 10% Ammonia solution added. Appearance of pink color precipitate indicates the presence of anthraquinones.

IR Spectrum

The FTIR spectrum of solid sample was obtained from ethanolic extract of plant leaves powder. To run the solution, a drop of the extract was placed on the face of a highly polished salt plate (i.e. 100mg of potassium bromide, Kbr). A second plate was placed on the top of the first plate so as to spread liquid in these layers between the dried plates and clamp the plates together in suitable fashion. Then the spectrum was obtained with the help of spectrophotometer and computer software attached with it[22]. The frequencies of different compounds present in the sample were analysed. The same procedure was followed for the suitable standard. The samples were run at infrared region between 400nm and 4000nm. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information for IR spectroscopic study.

RESULT

The present study subjected to screen the preliminary phytochemical constituents of two plants namely *Salvadora persica and Crescentia cujete* by qualitative tests.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Salvadora persica* and *Crescentia cujete* with two different solvents, viz., ethanol and aqueous extracts were presented (Table 1 & Table 2). The ethanolic extract of *Salvadora persica* showed the presence of compounds such as flavonoid, alkaloid, glycosides, terpenoids, phenols, anthraquinones. The aqueous extract showed the presence of compounds such as tannins, saponin, flavonoids, alkaloid, cardiac glycosides, phenols, coumarins. Some of the following compounds are totally absent in two extracts also such as carbohydrates, proteins, quinines, steroids, phlobatannins. Among two solvents, the leaf aqueous extract showed the presence of highest phytocompounds in *Salvadora persica* plant (Table1).

The ethanolic extract of *Crescentia cujete* showed the presence of compounds such as Tannins, flavonoids, quinines, glycosides, terpenoids, steroids. The aqueous extract showed the presence of compounds such as Anthraquinones, coumarins, terpenoids, cardiac glycosides, alkaloids, flavonoids, tannins. Some of the following compounds are totally absent in two extracts also such as carbohydrates, saponin, phenols, proteins, phlobatannins. Like *Salvadora persica, Crescentia cujete* has highest phytocompounds in aqueous extract while compared to ethanolic extract (Table 2).

FTIR

FTIR spectroscopy was useful for identification of functional groups, when run under IR region from 400 - 4000cm-1. There was a variation in the peaks of the sample which helps to determine the functional groups. Each peak represents single compound based on their density. The typical IR spectra for two plant extract are obtained and shown (Figure 1 and Figure 2). Figure 1 represents the IR spectrum of *Salvadora persica* leaf. In this spectrum 5 peaks are identified that are 599.89, 654.86, 1409.06, 1431.24, 2930.96. Figure 2 represents the IR spectrum of *Crescentia cujete* leaf. In this spectrum 13 peaks are identified that are 470.65, 520.80, 630.75, 652.93, 776.38, 1060.89, 1155.41, 1248, 1321.30, 1431.24, 1527.69, 2860.56, 2924.21.

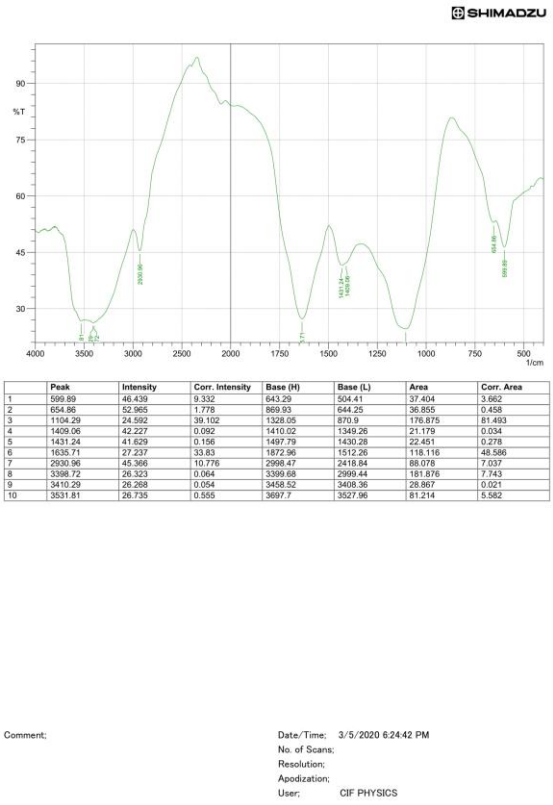
The broad absorption around 3797.84 cm -1and 2353.16 cm -1 the O-H stretching frequency is due to the presence of alcohols or phenols. Another prominent absorption around 2918.30 cm -1and the weak absorption such as 1371.39cm -1, 1325.10cm -1, is mainly due to alkanes (C-CH 3 stretching vibrations). In the spectrum, the medium range such as 1618.28 cm -1,1631.78 cm -1, is mainly due to amines (C=C stretching vibration). The C=O stretching vibrations at 1728.22 cm-1, due to the presence of esters or carboxylic acid or lactones. At 1442.75 cm -1, CH bending frequency is due to the aldehyde or ketones. Particularly below 1200 cm -1, C-O-C vibrations in ester at the range between 1100 cm-1 to 1200 cm -1 due to the presence of acetates or formats. At the range between 600 cm -1 to 700 cm -1, -CH-CH- bond frequency, is mainly due to the ethylene groups.

Table 1. Qualitative phytochemical analysis of different solvent extracts (Ethanol and Aqueous) of *Salvadora persica* (leaf)

Phytochemicals	Ethanol	Aqueous
Carbohydrates	-	–
Tannins	-	+
Saponin	-	+
Flavonoid	+	+
Alkaloid	+	+
Quinones	-	–
Glycosides	+	–
Cardiac glycosides	_	+
Terpenoids	+	–
Phenols	+	+
Coumarins	-	+
Proteins	-	–
Steroids	-	–
Phlobatannins	-	–
Anthraquinones	+	–

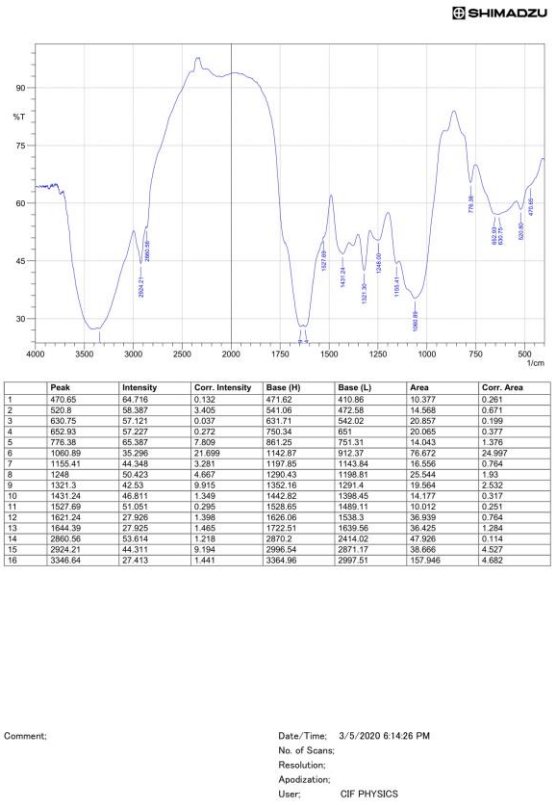
Table 2. Qualitative phytochemical analysis of different solvent extracts (Ethanol and Aqueous) of *Crescentia cujete* (leaf)

Phytochemicals	Ethanol	Aqueous
Carbohydrates	-	–
Tannins	+	+
Saponin	-	–
Flavonoid	+	+
Alkaloid	-	+
Quinones	+	–
Glycosides	+	–
Cardiac glycosides	_	+
Terpenoids	+	+
Phenols	-	–
Coumarins	-	+
Proteins	-	–
Steroids	+	–
Phlobatannins	-	–
Anthraquinones	-	+



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	599.89	46.439	9.332	643.29	504.41	37.404	3.662
2	654.86	52.965	1.778	869.93	644.25	36.855	0.458
3	1104.29	24.592	39.102	1328.05	870.9	176.875	81.493
4	1409.06	42.227	0.092	1410.02	1349.26	21.179	0.034
5	1431.24	41.629	0.156	1497.79	1430.28	22.451	0.278
6	1635.71	27.237	33.83	1872.96	1512.26	118.116	48.586
7	2930.96	45.366	10.776	2998.47	2418.84	88.078	7.037
8	3398.72	26.323	0.064	3399.68	2999.44	181.876	7.743
9	3410.29	26.268	0.054	3458.52	3408.36	28.867	0.021
10	3531.81	26.735	0.555	3697.7	3527.96	81.214	5.582

Fig 1. FTIR analysis of *Salvadora persica* (leaf)



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	470.65	64.716	0.132	471.62	410.86	10.377	0.261
2	520.8	58.387	3.405	541.06	472.58	14.568	0.671
3	630.75	57.121	0.037	631.71	542.02	20.857	0.199
4	652.93	57.227	0.272	750.34	651	20.065	0.377
5	776.38	65.387	7.809	861.25	751.31	14.043	1.376
6	1060.89	35.296	21.699	1142.87	912.37	76.672	24.997
7	1155.41	44.348	3.281	1197.85	1143.84	16.556	0.764
8	1248	50.423	4.667	1290.43	1198.81	25.544	1.93
9	1321.3	42.53	9.915	1352.16	1291.4	19.564	2.532
10	1431.24	46.811	1.349	1442.82	1398.45	14.177	0.317
11	1527.69	51.051	0.295	1528.65	1489.11	10.012	0.251
12	1621.24	27.926	1.398	1626.06	1538.3	36.939	0.764
13	1644.39	27.925	1.465	1722.51	1639.56	36.425	1.284
14	2860.56	53.614	1.218	2870.2	2414.02	47.926	0.114
15	2924.21	44.311	9.194	2996.54	2871.17	38.666	4.527
16	3346.64	27.413	1.441	3364.96	2997.51	157.946	4.682

Fig 2. FTIR analysis of *Crescentia cujete* (leaf)

DISCUSSION

Phytochemical analysis

Seeds bark and leaves of *Salvadora persica* are reported to phenolic compound[23].Similarly the ethanolic extract of *Salvadora persica* showed the presence of phenols in our study. *Salvadora persica* contained small amount of saponin[24]. Fruits contain large amount of sugar, and alkaloid from the seeds. In our study aqueous and ethanol extracts both showed the presence of compounds like saponin, alkaloids. But trace amount of sugar (carbohydrates) may be present in crude extract, but it is not responsible to preliminary phytochemical analysis. Several research are going on this particular species around the world. Three lignan glycoside compounds are identified[25]and flavonoid compounds such as rutin and quercetin were detected in the stems of *Salvadora persica*[26]. In overall, crude extract contains the same compounds in both extracts.

Phytochemical study of *Crescentia cujete* fruits are yielded saponins, flavonoid, cardenolides, tannins, and phenol (Stuartxchange). The leaves extract of *C. cujete* and *C. alata* showed glycosides compounds[27]. Several Anthracene derivatives compounds are coumarins, lignans, triterpenes, steroids mono and diterpenes, anthraquinones that are obtained from the methanolic extract of leaves of *C. cujete*[28], similarly the related genus *Kigelia pinnata* showed the same phytocompounds[29]. Like *Salvadora persica, Crescentia cujete* has highest phytocompounds in aqueous extract while compared to ethanolic extract.

FTIR analysis

FTIR spectroscopy was useful for identification of functional groups, when run under IR region from 400 - 4000cm-1. There was a variation in the peaks of the sample which helps to determine the functional groups. Each peak represents single compound based on their density. The typical IR spectra for two plant extract are obtained. The IR spectrum of *Salvadora persica* leaf revealed 5 peaks that are identified as 599.89, 654.86, 1409.06, 1431.24, 2930.96. The IR spectrum of *Crescentia cujete* leaf revealed 13 peaks that are identified as 470.65, 520.80, 630.75, 652.93, 776.38, 1060.89, 1155.41, 1248, 1321.30, 1431.24, 1527.69, 2860.56, 2924.21.

In both plants, IR spectrum showed the broad absorption around 3797.84 cm -1and 2353.16 cm -1 the O-H stretching frequency is due to the presence of alcohols or phenols. Another prominent absorption around 2918.30 cm -1and the weak absorption such as 1371.39cm -1, 1325.10cm -1, is mainly due to alkanes (C-CH 3 stretching vibrations). In the spectrum, the medium range such as 1618.28 cm -1,1631.78 cm -1, is mainly due to amines (C=C stretching vibration). The C=O stretching vibrations at 1728.22 cm-1, due to the presence of esters or carboxylic acid or lactones. At 1442.75 cm -1, CH bending frequency is due to the aldehyde or ketones. Particularly below 1200 cm -1, C-O-C vibrations in ester at the range between 1100 cm-1 to 1200 cm -1 due to the presence of acetates or formats. At the range between 600 cm -1 to 700 cm -1, -CH-CH- bond frequency, is mainly due to the ethylene groups.

Similarly, FTIR spectrum analysis of *Caralluma fimbriyata* revealed the peak value at 2970.38 and 2885.51 cm^{-1} refers to the presence of alkanes (C–H stretch). The peak at 1759.08 and 1666.50 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak of 1327.03 cm^{-1} showed the presence of aromatic amines (C–N stretch). The peaks of 1273.02, 1087.85 and 1049.28 cm^{-1} indicate the alcohols, carboxylic acids, esters, ethers (C–O stretch). A peak of 879.54 cm^{-1} revealed the alkenes (=C–H bend). These basic reports are used to find out found the presence of phenols, alkanes and aromatic amines[30]. Same absorption spectrums are obtained in extract of *Aerva lanata*[31], *Albizia lebbeck*[32] and *Ardisia blatteri*[33].

CONCLUSION

Salvadora persica is a large shrub or a small tree. The plant is highly medicinal plant. Moreover, trees are often cultivated for roots as it is extensively used in preparation of Meswak toothpaste. The tree possesses multiple medicinal benefits. *Crescentia cujete* is otherwise called as Calabash tree. This plant is widely cultivated as an ornamental shade or specimen tree in tropical areas. This plant has been used as medicinal plant since ancient times. *Crescentia cujete* possessed various therapeutic activities in related to findout drug in pharmacological aspects. Over exploitation over the last few decades have threatened the existence of both important multipurpose trees (*Salvadora persica* and *Crescentia cujete*). These plants need urgent restoration and conservation that is severely required.

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