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PHARMACOGNOSTIC EVALUATION OF THE LEAF OF *Rhus succedanea* VAR. HIMALAICA. J. D HOOKER.

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Abstract

Background: *Rhus succedanea* is generally traded, distributed and sold in the markets in its crude and raw form. This may have been mixed with adulterants, mismanaged by malpractices and substituted with other closely related drugs having different effect. This study is therefore carried out to authenticate the plant through pharmacogonostic evaluations.
Material & Methods: The organoleptic studies were carried through sensory organs i.e size, shape, texrure, odour, etc. Histological studies were conducted by preparing hand slides, mounting the specimen in potato tuber; fluorescence characters were determined through UV and phytochemical screening was investigated using various standard and common methods from relevant literature.
Results: Morphologically, the *Rhus* is a perennial small sized deciduous tree, 5–9 m tall with opposite imparipinnately compound leaves and small grayish yellow flowers born on paniculate inflorescence; locally, called as Rakhkal in Pashto and Kakarsingi in Urdu. The organoleptic evaluation showed leaf had pleasant, aromatic odour and astringent taste. Transverse section of leaf through midrib region was worked out. The anatomy of the midrib has shown to be surrounded by both upper and lower epidermis with multicellular non-glandular trichomes. The leaf was hypostomatic showing anomocytic stomata with average stomatal number 27.1 ± 7.2 and stomatal index 14 ± 3.63. The average vein islet, vein termination and palisade ratios were 13.6 ± 3.04, 10.21 ± 1.92 and 6 ± 2.01 respectively. Leaf powder showed the existance of anomocytic stomata, spirally thickened xylem vessels, non-glandular multicellular and stellate trichomes. Fluorescence study and percent extractive values was also carried out. The phytochemical screening showed the presence of carbohydrates, protein, alkaloids, phenols, flavonoids, terpenoids and anthraquenones, while tannins and fixed oil was not detected. Quantitatively highest amount of alkaloids 16% and flavonoids 19% in leaf was detected.
Conclusion*:* The results of the of the anatomical, organoleptic and physiochemical studies of the powder of leaf will be helpful in standardization of *R. succedanea* the crude drug.

Key Words: Pharmacognostic evaluation; leaf; *Rhus succedanea;* organoleptic evaluation; Anatomy; Fluorescence study; phytochemical screening;

Introduction

Pharmacognosy is a multidisciplinary field that comprises organoleptic, botanical, physical, chemical, biological and pharmacological considerations for the study and evaluation of crude drugs from natural sources (Selvam, (2015).

The plant crude drugs are generally traded, distributed and sold in the markets in their crude and raw form which is the main source for production, formulation and synthesis of natural drugs. This may have to be mixed with adulterants, mismanaged by malpractices and substitute with other closely related drugs having different effect.

So the pharmacognostic (organoleptic and macroscopic) and botanical characterization was necessary to get the desired and genuine drug for good medicinal effect. The basic aim of pharmacognosist was to judge the importance of natural crude materials to the required standard by passing through strict standard procedures of pharmacognosy.

That reduces the wrong recommendations of medicinal plants and traditional medicines to high extent (Kumar, (2007). The stepwise pharmacognostic investigations, including morphology, anatomy, quantitative microscopy determination such as stomata number, index, vein islet r, veinlet termination number, palisade ratio and qualitative and quantitative phytochemical screening give standardization of the crude drugs. Correct identification, authentication and quality assurance of the preliminary resources as an important requirement to make sure the reproducible quality of phytomedicine which will show the safety and effectiveness of herbal products (Shweta et al., (2012). Extractive values determine different types of active phytoconstituents and its amount in medicinal plants on the bases of its nature and solvent used (Chetai & Gogoi, (2011).

Fluorescence analysis is an important consideration for 1 st line standardization of therapeutic crude natural drugs. It was carried out by observing the drug/powder or extract dissolved in certain solvent and observing under UV light 254 nm, 365 nm and visible light (Kadam et al., (2012).Phytochemicals make the value of medicinal plants by altering of a definite physiological action on the human body function and are new anti-infective agents from plants (Juliet et al., (2012).

Evaluation of the chemical determination and quantification of crude drugs was not only for discovering pharmaceutical agents, but might be also a significance in revealing new sources of low cost homoeopathic materials which were used in production of synthetic complex chemical substances treating severe illnesses and gives us clues to the discovery of new valuable Drugs (Badugu, (2012).

Materials and methods

Collection and Preservation

The fresh leaves specimens *Rhus succedanea* var. *Himalica* were collected from Shalmanay Kotkay, Disttrict Shangla. Some ecological and morphological characteristics were recorded at the time of collection in their natural habitat including altitude, size of tree, phyllotaxes,type, etc. plant sample were collected at flowering season, taken to the Herbarium for correct identification by Curator Mr. Ghulam Jelani department of Botany University of Peshawar. The Samples were air dried, mounted on Herbarium sheet and provides Voucher specimen number Bot. Khan. (2007) (PUP) and deposited in the herbarium.

Morphological observation

The morphological observation of leafof *R. succedanea* L.following method of (Wallis, 1985).

Macroscopic studies

The macroscopical study were carried out organoleptically by observing, Shape, Colour , Size, Odor, Taste, Surface, Surface fracture, Texture, Apex, Type, Venation, Leaf margin etc

Microscopic Study

The microscopic study and transverse section of the leaves of *R. succedanea* L. was done by the procedure of (Tajuddin et al., (2013); Solanki et al., (2011).

Histology

The histological study of the *R. succedanea* leaf was done with hand sectioning using method of (Johnson & Johnson, 2006; Okhale et al., (2010).

Leaf surface study

The following leaf surface feature were studied

a) Stomatal Number and Stomatal Index: Stomatal number (SN) or stomatal density is define as the average number of stomata count in 1 mm square of the leaf within both upper and lower epidermises. Stomatal index (SI) is the percent ratio of stomata to the total number of epidermal cells in 1mm square area (Evans, (2002); Xavier et al., (2015).

Procedure: For determination of stomatal number and stomatal index within both upper and lower epidermises were peeled off from fresh young leaves using a pair of forceps, razor and by sticking transparent cotton tape. The peeled section was fixed on slide by glycerin and examined under Labomid digital microscope using 100X magnification. Numbers of stomata per mm square were recorded for stomatal number. The following standard formula was used to calculate to the stomatal index.

$$I = \frac{S}{S + E}$$

I= Stomatal Index S= Number of stomata per mm square E= Number of epidermal cells per mm square

b) Veinlet Termination and Vein Islet Number: Veinlet termination number is the determination of average number of terminated veinlet in 1 mm square of leaf surface area taken from region of midrib to margin of the leaf Veinlet termination the ultimate free end of veinlet. Vein islet number is the average number of veinlet enclosing small green area in 1 sq mm leaf surface (Wallis, 1985; Evans, (2002); Omoregie et al., (2015).

Procedure: Lamina of leaf between midrib and margin was cut into small pieces about 1-3 mm square and boiled in a concentrated solution of chloral hydrate for 15 minutes till the discoloration of pieces. The transparent fragments were transferred into glass slide and observed under microscope at magnification of 10X. Vein islets were counted in 1mm sq area. Along with veins-islets the veinlet terminations were also, counted which were inside the square only. To calculate exact, accurate and standard values five readings were taken for each vein islet and vein termination number, and the slides were photographed (Evans, (2002); Hedge et al., (2015).

a) Palisade ratio: Palisade ratio is defined as the average number of Palisade cells below single upper epidermal cell (Evans, (2002). It is an important parameter for determination and characterization of leafy drugs (Barkatullah et al., (2015).

Procedure: Small pieces (1-2mm) of leaf grown in full sun light were taken and cleared by boiling in 200% Chloral Hydrate solution. The cleared pieces were mounted and examined under microscope. A number of groups of each of four upper epidermal cells were first focused. Then by minor rotation of the fine adjustment, the under lying palisade cells were focused within the area of four epidermal cells. Palisade ratio was then obtained by dividing the number of palisade cells by 4. Five readings were taken from different pieces in order to obtain accurate average.

Investigation of powder Assessment of the powdered drug for detection of various types of cells, tissues, starch granules and calcium-oxalate crystals, vascular tissues, stomata etc., was conducted by most commonly used method of (Sailor et al., (2010) as given below,

Procedure: Dried leaf powder was passed through fine sieve (no. 60) and boiled in concentrated chloral hydrates for 15 minutes, macerated in glycerin and iodine solution on glass slide and observed under compound microscope for various all structures.

Fluorescence Analysis Fluorescence characteristics of the powder were observed by method of **(**Ozcan et al., (2011). **Procedure:** A small amount of powder was macerated in a particular reagent mention in (Table.3) for 5 minutes and was observed in visible light as well as under UV lamp in both wavelengths (short 256 nm and long 360nm) for fluorescence. **Extractive Values Determination.** 10 gram of the crude powder drug of each leaf of *Rhus succedanea* was dissolved in 200ml of a respective solvents and keep in air tighten bottles for five days with intermittent shaking. Each extract was filtered into a pre-weighted flask. The solvents were than evaporated and the flasks were again weighted to know weight of the extract. The solvent used for extractive values were n-butane, Chloroform, Acetone Methanol, Ethanol and Distilled Water arrange according to polarity index. Determination of % extractive valves was carried out by the following formula

$$\text{Percent (\%) extractive value } \left(\frac{W}{W}\right) = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} X\,100$$

Preliminary Qualitative Phytochemical Screening: For the detection of the phytochemicals various screening tests were performed as given below.

Tests for Carbohydrates

Benedict test: (Alkaline solution containing cupric complex) 2ml of an extract was dissolved in ethanol and equal amount of Benedict's reagent was added drop wise and boiled on water bath. The formation of Brick red or reddish brown precipitation will indicates presence of carbohydrates (Evans, (2009).

Fehling test (Copper sulphate in distilled water) To 1ml of the extract 1ml of Fehling's A and Fehling's B was (Potassium tartarate and sodium hydroxide in distilled water) and boiled on spirit lamp. A characteristic colour change due to formation cuprous oxide will shows the presence of reducing sugar in the extract (Evans, (2009) and Ozcan et al., (2011).

Proteins and amino acids detection test

Ninhydrin method: (Indane 1, 2, 3 trione hydrate) To 5ml of extract 0.2% Ninhydrin solution was added and boiled in test tube. The formation of violet colouration will show presence of protein and amino acids (Edeoga & Eriata, (2009)

Biuret test: 2 ml of plant extract was treated with few drops of copper sulphate solution. Then to this 2ml of each ethanol and potassium hydroxide were added. The appearance of pink colour will shows existence of proteins (Edeoga & Eriata, ((2009)) and Hegde et al., (2015).

Xanthoproteic test: 10 ml of the extract was mixed with several drops of concentrated HNO3.The appearance yellow colour will be the sign of presence of protein (Edeoga & Eriata, (2009).

Tests for Alkaloids

Mayer's test: (Potassium mercuric iodide solution). To 30 ml of extract ethanoloic solution, Mayer's reagent was added drop wise. Creamy white precipitate will show presence of alkaloids (Deore et al., (2015).

Wagner's test: (solution of Iodine in Potassium Iodide). 10ml of sample extracts was treated with few drops of Wagner's reagent through dropper, appearance of red brown precipitation will be the sign alkaloid existence (Deore et al., (2015).

Phenol Detection Test

Ferric Chloride To 10ml extract solution, few drops of FeCl3 solution were added. Appearance of bluish black or green colour will show presence phenols (Chavre, (2015).

Flavonoids detection test

Alkaline reagent test: NaOH solution was added to 20ml of plant extract solution. Formation of yellowish red precipitation shows presence of flavonoids (Badugu, (2012).

Lead Acetate test: Plant extract was treated with few drops of lead acetate solution. The appearance of yellow colour precipitation in the solution will indicate the presence of flavonoids (Onocha et al., (2011).

Fixed Oil detection test Powder extracts of leaf of the selected plant were keep and pressed in between filter paper, appearance of permanent greasy spots on the filter paper will be the indication of presence of fixed oil (Onocha et al., (2011); Hegde et al., (2015).

Saponin detection test

Frothing test:5ml of the extract solution was taken in a test tube and shaken vigoursly. Froth formation will indicate the presence of saponin (Chaouche et al., (2011).

Foam test 3 gram of the extracts was dissolved in 20 ml of distilled water and was shaken vigorously for 15 minutes. Appearance of permanent foam for more than 10 minutes will indicate the presence of saponin (Tiwari et al., (2011).

Hydrochloric acid test 5ml of extract sample solution was treated with few drops of HCl. Appearance of pinkish red, which on addition of ammonia solution changed into deep violet shows existence of saponins (Harborne, 1998).

Test for detection of Terpenoids

Salkowski's test: 2g of extract solution was mixed with several drops of chloroform and H2SO4. Appearance of red colour in lower portion will show presence of terpenoids (Harborne, 1998).

Copper acetate test: Extract was dissolved in distilled water and 4-5 drops of copper acetate solution was added. The formation of green emerald colour will indicate presence terpenoids (Tiwari et al., (2011).

Detection test for Tannins

Ferric chloride test 5ml of FeCl2 solution was added to 10ml of extract solution. The formation of bluishback precipitate will show occurrence of tannin (Somkuwar and Kamble, (2013).
Alkali reagent test 10 ml plant extract solution was mixed with NaOH. Formation of yellow-red precipitation quickly indicates presence of tannins (Somkuwar and Kamble, (2013).

Detection test for Anthraquinones
Borntrager's Test 10 ml of extract was mixed with 10% FeCl2 solution and heated, to which 2ml of pure hydrochloric acids were added and filtered. Filtrate was allowed to cool and then shaken with diethyl ether. Then concentrated ammonia solution was added. The appearance of pink or deep red colouration of aqueous layer will indicates the presence of anthraquinone (Niratker and Sailsaja, (2014).

Quantitative chemical analysis

Quantitative determination (amount & percentage) of phytochemicals like Alkaloids, flavonoids and phenols was carried out for ethanolic extract of leaf of *R. succedanea*.

Total Alkaloids determination Total percent alkaloid of leaf of *R. succedanea* was determined by standard method of Harborne, (1998).

Procedure

100 ml of 10 percent acetic acids solution was added to the 2g ethanolic extract in a beaker and kept for four hrs covered with aluminum foil. The solution was then concentrated to 1/4th of its original volume by evaporating on water bath and concentrated NH4OH, was added. Formation of precipitate occurred, which was collected on pre weighted (W1) Whatman filter paper and then thoroughly washed with dilute ammonium hydroxide. The residues along with filter paper was dried, weighed (W2) and amount of alkaloid in mg/g as well as percentage was calculated as,

$$\text{Amount of Alkaloids} = \frac{X}{\text{Weight of Sample}}$$

$$\text{Percent Alkaloids} = \frac{X}{\text{Weight of Sample}} X\ 100$$

Where
X= Weight of alkaloids = W2- W1 W1= Weight of filter paper
W2 = Weight of filter paper+ precipitate

Total flavonoids determination

The total flavonoids of leaf extracts were carried out using the Boham & Kocipai. 1994; Mir et al., (2013).
Procedure Plant parts (5 g) extracts were dissolver in 100ml of 80% aqueous methanolic extracts and keep overnight in refrigerator. On next day add chloroform to the solution (for glycosides flavonoids) or ethyl acetate (for aglycosides flavonoids) drop wise and transfer to the pre-weighted beaker (W1), placed on water bath, evaporated to dryness and weighted (W2). The amount and percentage was calculated as

$$\text{Percent Flavonoids} = \frac{X}{\text{Weight of Sample}} X\ 100$$

Where
X= Weight of flavonoids = W2- W1 W1= Weight of Beaker
W2 = Weight of beaker+ remain

Total Sterols determination: Total percent sterols of leaf were determined by standard method of **(**Boham & Kocipai, 1994; Kokate, (2008).

Procedure: For determination of percent sterols 2 g of the extract was dissolved in 75 ml of distilled water and 30ml of 10% KOH solution
were added. The solution was then transferred into separating funnel and extracted thrice with 75 ml petroleum ether each time. From each extraction the ether layer was transferred into the pre-weighted beaker (W1) and keep on water bath to completely evaporation of the solvent. The sterol content remains in bottom, weight the flask along with contents (W2) and the amount and percentage was determined using the following formula (Huang *et al*., (2010)).

$$\text{Amount of Sterol mg/g} = \frac{X}{\text{Weight of Sample}}$$

$$\% \text{ Sterols} = \frac{X}{\text{Weight of Sample}} X\,100$$

Where
X= Weight of sterols = W2- W1 W1= Weight of beaker
W2 = Weight of beaker+ contents

Results and Discussion

The pharmacognostic evaluation of leaf of *Rhus succedanea* var. *himalaica* J. D. Hooker family anacardiaceae was carried out that include morphology, macroscopy, microscopy, physiochemical and phytochemical analysis.

Morphology

The morphological observations of the plant was carried out on the spot at the time of collection, which showed that the plant is a perennial small sized deciduous tree, of about 5–9 m tall, locally referred to as Rakhkal in Pashto and Kakarsingi in Urdu. The plant produces latex on injury which is considered to be highly toxic and allergic, causing severe dermatitis to local inhabitants, whenever the body of a person comes in contact with the plant or its latex. Susceptible peoples can require temporary hospitalization, although other people are immune. *Rhus* has imparipinnately compound leaves, arranged oppositely with inflated petiole, entire margins and aristate apex. The stem was thick glabrous, branched and having thick bark producing white latex on injury. The roots large tape root extensively branched, showing secondary growth. The flower was mall grayish yellow in colour forming paniculate inflorescence. For further study the leaf and root were selected. Rakholiya & chanda; (2012) Sher et al, (2011);
Gunoz et al. (2005) worked out *Mangifera indica*L. var. Kesar (Anacardiaceae)., *Pavonia Odorata* & *Linaria corifolia* which are in line the presenr observations.

Macroscopy and Organoleptic characteristics

Macroscopic and organoleptic (sensory) evaluations are the main features in standardization and identification of crude natural drugs and the only parameters that required no involvement of scientific instruments neither any expenses. Morphological, microscopical and physical evaluation gives valuable simplest, quickest and easiest information to institute purity and quality regarding the characteristics and recognition of crude drugs (Rakholiya & chanda, (2012); (Zongo et al., (2013). (Naghibi et al., 2005). Macroscopical investigation of the fresh and dried leaf of *Rhus succedanea .var himalaica* was carried out. The macroscopical observation of the leaf were carried out and listed in (Table.1). The macroscopy shows that *Rhus* have imparipinnatly compound leaf in which the leaflet were oppositely arranged. Leaflet was green on upper dide and pale green at lower side when fresh and became light green or greenish brown on shade drying (Figure 1 & 2). The shape of leaflet was lenceolate, 4-14cm in length, 3-6cm in width, with inflated petiole, entire leaf margins, aristate apex, unicostate reticulate venation and having smooth surface showing no presence of trichomes (Figure 1 & 2). The odour and taste of the leaf was pleasant, aromatic and astringent. Similar studies were conducted by various workers which are in support with present work. Ibrahim et al. (2015) conducted macro-morphological roots of *Agemone Mexicana* L. and documented the size (7-32cm), cylindrical in shape, grey-brown colour with short fracture and the fracture surface is rough. Shweta et al. (2012) registered that the leaf of *Rivea hypocrateriformis*was green in color, orbicular-cordate shape in shape, 3-8cm length, smooth margin and bitter taste. Other various workers i.e, Juliet et al. (2012); Madhavan et al. (2010); also carried out the macro-morphological evaluations of certain plants like *Didymocarpus tomentosus* Wight., *Pavonia Odorata* and *Nothosaerva brachiata*and and concluded that the morphological evaluations provide a base for the standardization of drugs and also give authentic parameter for taxonomic and systematic characterization. The present study on *R. succedanea* explored numerous characters of plant, to the taxonomist for its in deep taxonomic study and to work out intra generic differentiation.

Fig. 1 & 2 Fresh and Dried leaf of *Rhus succedanea .var himalaica*

Table 1: Morphological and Organoleptic evaluation of *Rhus succedanea* leaf.

Plant Parts	Features	Fresh	Dry
Leaf	Size	Length= 4-14cm; width; 3-6cm	Length= 4-14cm; width; 3-6cm
	Leaf shape	Lanceolate cordate	Lanceolate cardate
	Color	Upper side green; lower pale green	Both surfaces brownish green
	Odour	Pleasant	Pleasant
	Taste	Astringent	Astringent
	Petiole	Inflated	Inflated
	Incisions	Entire	Entire
	Composition	Imparipinnately compound	Imparipinnately compound
	Venation	Reticulate unicostate	Reticulate unicostate
	Leaf base	Cordate	Cordate
	Leaf Apex	Aristate	Aristate

Microscopy and histology: For Miscroscopical and histological features of leaf of *R. succedanea* the transverse sections were prepared, stained and through compound digital Labomid microscope pharmacognosy Lab University of Peshawar and photographs were taken (Figures 3 & 4).

Leaf anatomy and Histology: The Transverse section the leaf of *R. succedanea* through midrib region appeared fusiform shape and showed the presence following tissues under light microscopy (Figure 3).
Epidermis: Anatomy of the midrib has shown to be surrounded of both upper and lower epidermis comprises closely and compactly arranged uniseriate cells which are further surrounded by smooth thin transparent cuticle. The upper epidermis gives rise to various long multicellular non-glandular trichomes.
Cortex: Both the epidermises were followed by 3-5 layered of cortex, consisted of large thin walled isodiametric cells. Cortex showed the presence of latex ducts.
Pericycle: The cortex was followed by a single layered pericycle entailed with small spherical cells that surrounded the vascular bundles in the midrib region.
Vascular Bundles: The pericycle continued by intermix vascular bundles that comprises a stalk of xylem vessel arranged one above the other. Primary xylem located towards the center while phloem was towards the outer side and phloem parenchyma was intermixed with xylem vessels.
Pith: The center of the midrib was occupied by large central parenchymatous pith region composed of thin walled large irregular shaped cells. Anatomy of the leaf of *R. succedanea* through lamina showed that the upper epidermis is followed by **palisade parenchyma** that comprises long tubular, cylindrical, columnar cells, which are compactly arranged in single layer. Below the palisade parenchyma and above the lower epidermis, the lamina was consisted of **spongy parenchyma** that showed the presence of polygonal loosely arranged and hexagonal cells with many large intercellular spaces (Figure 4). The following investigators worked on leaves of various plants, and our results are in line with these workers. Rakholiya & chanda, (2012) worked on leaf of *Mangifera indica*L. var. Kesar (Anacardiaceae). Admani et al.((2015) reported that *Woodfordia fruiticosa* leaf shows a typical dicot anaotomy. Various other researchers like Goswami, (2015); Xavier et al. (2015); Tajuddin et al. (2013); Khyade and Vaikos, (2014)); Gupta & Rao, (2012); Amponsah et al. (2014) worked on leaves of various plants i.e, *Catharanthus roseus* (L.) *Homonoia riparia*, *Dioscorea hispida* Dennst., *Wrightia tinctoria*, *Fumaria indica* (Hausskn.) *Ocimum gratissimum*, *Hilleria latifolia* and reported somw what similar results, and recommended that microscopy gives authentic information about the identification of plant and provide a base for standardization of crude leafy drugs.

Leaf surface featues: The leaf suraface features of *R. succedanea* leaf was acarried out that comprising stomatal studies, vein islet number, vein termination number and palisade ratio.
Stomatal Studies Stomata are pores in the epidermis and made by pairs of architecturally and physiologically specific guard cells and neighboring epidermal cells known as subsidiary cells. This specialized group of cells form stomatal complex that accelerates exchange of gases between plants and external environment. Stomatal study of the leaf showed that the leaf was hypostamatic, i.e the stomata were found on lower surface only and composed of only anomocytic type (the stomata surrounded by varying number of subsidiary cells, which have no special arrangement) spread all over the lower epidermis (Figure 5). The epidermis was composed of polygonal axially elongated cells, closely fitted by mix wavy and straight walls and covered with by thin layer of smooth cuticle. The numerical values range, mean and standard deviation per mm2 of stomatal numbers and stomatal index was recorded (Table. 2). The stomatal number ranged from 15-35 with the 27.1 ± 7.2 averages and standard deviation respectively. The stomatal index was from 10.2 - 19.9 rage and (14 ± 3.63) mean and standard deviation (Table. 2 & Figure. 6). The upper epidermis shows no presence of stomata and was comprising of polygonal axially elongated epidermal cells (Figure 6). The epidermal cells were covered by wavy thick walls and further the upper epidermis was protected by smooth transparent single layer cuticle (Figure 6).
Vein islet and vein termination number: Vein islet number is the average number of veinlet enclosing small green area in 1 square mm of leaf surface and Veinlet termination number is the determination of average number of terminated veinlets in 1 mm square of leaf surface area taken from region of midrib to margin of the leaf (Wallis, 1985; Evans, (2002); Omoregie et al., (2015). Vein islet and vein termination number of leaf was recorded and given in Figure 7 & 8, Table. 2. The range, mean and standard deviation was 10 – 19, 13.6 ± 3.04 and 8 – 14, 10.21 ± 1.92 respectively.

Palisade Ratio: Palisade ratio is the average number of Palisade cells below four upper epidermal cells (Chavre, (2015). It is an important parameter for determination and characterization of leafy drugs Barkatullah et al. (2015). The palisade ratio of *R. succedanea* was recorded from the transverse section of leaf lamina (Figure 4) and the numerical data was recorded in the form of range, average ratio and standard deviation (Table. 2). The results of palisade ratio range, average ratio and standard deviation were 8-13 and (6 ± 2.01) respectively (Table. 2 & Figure 4). Various other workers have also reported similar studies as given below. Gowdhami & Rajalakshmi. ((2015); Karthikeyan et al. (2012); Tripathi & Mondal, (2012); Khan et al. (2011); Bhogaonkar & Chavhan, ((2015)); Chavre, ((2015)); Amponsah et al. (2014) Kavian, ((2008)) worked on various quantitative parameters of leaf of *Jasminum, Abutilon indicum*.L, *Amaranthus viridis* Linn., *Cadaba fruticosa* (L.)., *Hilleria latifolia, Wattakaka Lilium ledebourii* and reported significant results Mbwambo et al., (2009); Ghimare et al. (2012); Janke & Dearmond. (2004) stressed that epidermal and cuticular traits of plants epidermal cells, type and arrangement, number, size of stomata, and shape of trichomes serve as vital tools in solving taxonomic problems in angiosperms (Tehseen et al., (2010). The stomatal diversity in the foliar epidermis has great value in plant systematics studies (Gupta et al., (2012). Stomatogenesis has long been studied by morphologists, physiologists and taxonomist and considered to be most important role in intragenic systematics and can be used as a taxonomic character for intraspecific diffentiation (Tripathi & Mondal, (2012). Vein islet number, vein termination number and palisade ratio are most important and authentic tools for differentiation among closely related species of the same family (Mbwambo et al., (2009).

Table 2: Leaf Surface features of *R. Succedanea.*

S.NO	FEATURES	Range	Average
1.	**Stomatal Number**	15 – 35	27.1 ± 7.2
2.	**Stomatal Index**	10.2 - 19.9	14 ± 3.63
3.	**Vein Islet Number**	10 – 19	13.6 ± 3.04
4.	**Vein Termination Number**	8 – 14	10.21 ± 1.92
5.	**Palisade Ratio**	8-13	6 ± 2.01

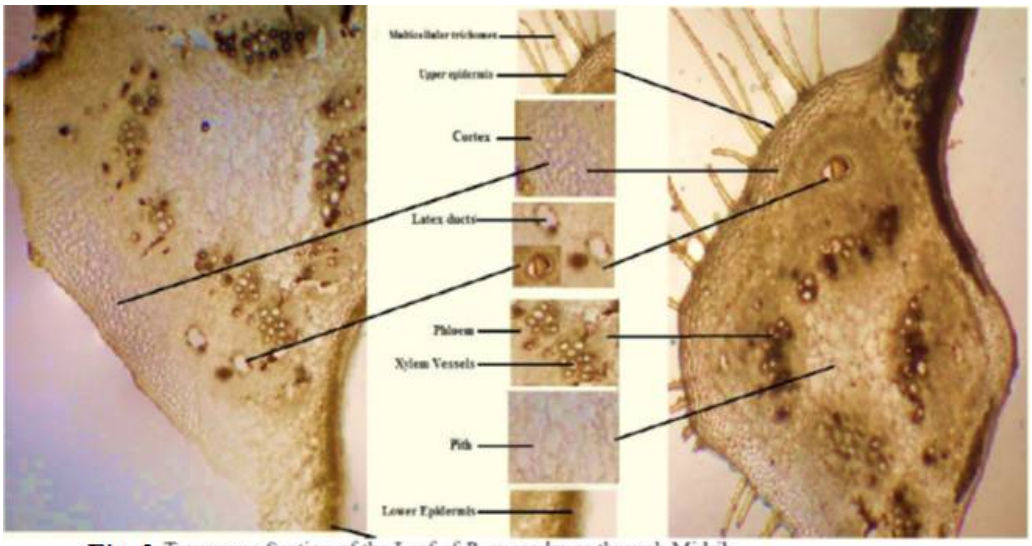

Fig. 3 Transverse Section of the Leaf of *R. succedanea* through Midrib

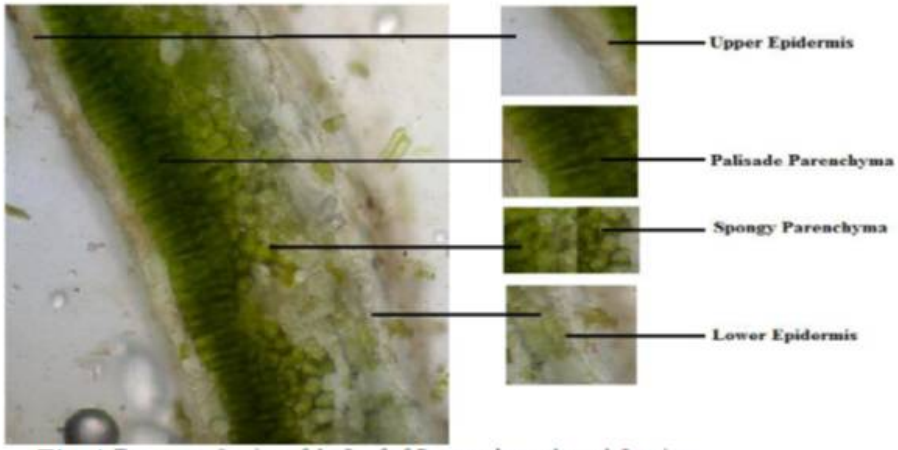

Fig. 4 Transverse Section of the Leaf of *R. succedanea* through Lamina

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Fig. 5 Lower epidermis of the leaf of *R. succedanea* showing Anomocytic type of stomata keys, St= Stoma, G.C= Guard cells, S.C= Subsidiary cells

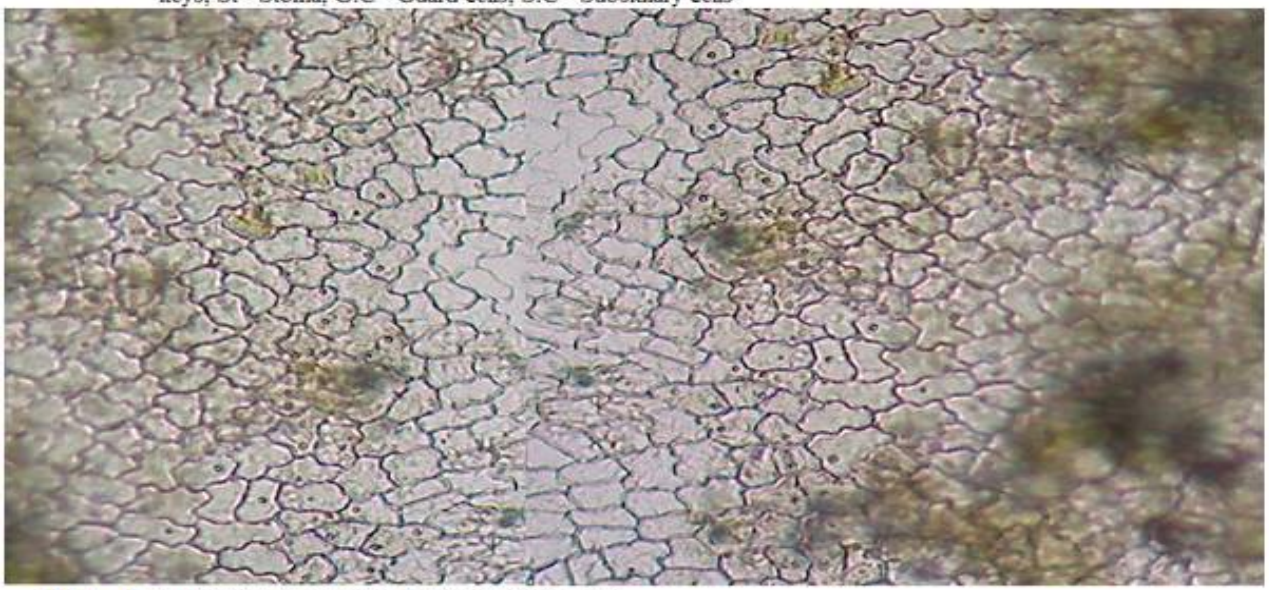


Fig. 6 · Upper epudermis of the leaf of R. succedanea

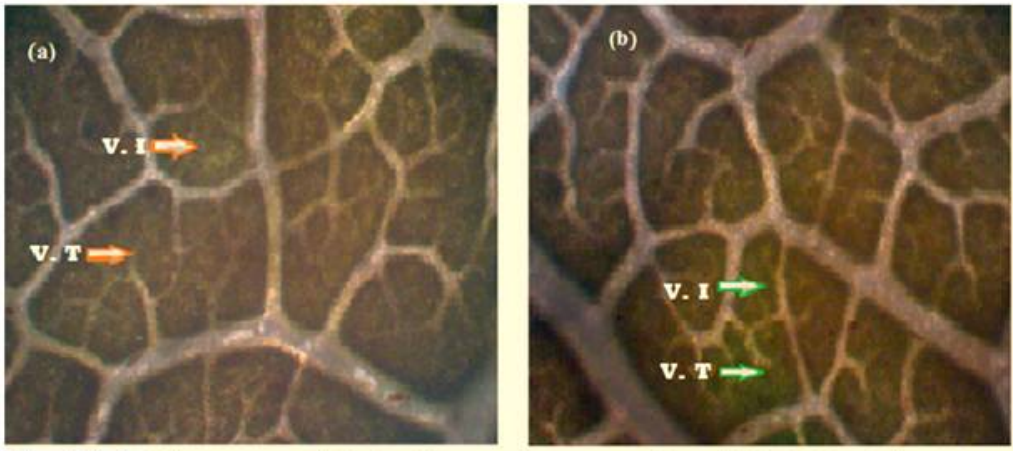


Fig. 7 & 8. Vein arrangement in lamina of *R. succedanea* representing vein islet (V. I) and vein termination number (V. T)

Powder drug study: The examination of powder drug of leaf of *R. succedanea* under microscope showed the existance of non-glandular unicellular trichomes, upper epidermis with anomocytic stomata. The powder exhibited the presence of fragments of upper epidermis having no stomata that showed that the plant is hypostomatic. Helical to Spirally thickened xylem vessels, cortex parenchyma cells, non-glandular multicellular trichome, stellate or star shaped trichomes, and spongy parenchymatous cells were also observed in crude powder of leaf (Figure 9 a-i). Similarly Omoregie et al. (2015); Pandavadra & Chanda, ((2014); Xavier et al. (201); Samanta et al. (2013); Sasmal et al., (2012); Saleem et al. (2010); Dinakaran et al. (2011); Solanki et al. (2011) and Juliet et al. (201) strongly supported the present findings as they did the powder microscopy of *Memecylon umbellatu*, *Crotalaria juncea* L., *Calotrapis proceraw*, *Homonoia riparia*, *Psidium guajava*, *Coccinia indica* & *Saraca asoca* Roxb. Resepectively and reported several similar tissues. Powder microscopy help in the identification of the herbal drugs and detection of adulteration in crude drugs (Soni et al., (2011).

Flourescence analysis: The fluorescent color was definite for chemical substance and remains an adequate sensitive procedure that enables the precise and accurate determination of pharmaceutical samples (Ozcan et al., (2011)). The leaf powder drug of *R. succedanea* were studied under visible and ultra violet, short (254nm) and long wavelength (366nm) light for fluorescence characters treated with different reagents like, Iodine, Picric Acid, (FeCl3), NH3 solution, NaOH, HCl, 50% HNO3, acetic acid and H2SO4. The powders showed various shades of color like black, brown, green yellow, red to brown black, dark black, yellowish brown, pink etc. with each reagent which was an indication of the presence of different chemical compounds and fluorescent substances (Table. 3). Many researchers worked out similar study on various and detected same type of variations in colors. Chand et al. (2012) reported that fluorescence is an essential tool to detect all ingredients in powders on reaction with various chemical mixtures under Ulta Voilet light. Biswal et al. (2011) also have similar statement, that fluorescence is important to detect the presence of phytoconstituents and fluorescent compound in crude powder when shows color changing with UV and various reagent. Ravikumar, (2011) reported that fluorescence can be used as diagnostic tool for detecting adulteration, if any. Similar study was also conducted by Vogel-Mikuš et al. (2009) for analysis of the powder of leaves of *Acacia modesta*. Kadam et al. (2012) reported that fluorescence study is an important feature of pharmacognostical evaluation for
preliminary standardization of powder drugs. Wallis, (2005) documented that the UV light is very active in generating fluorescent lumination in specific chemical compounds that donot show illumination in visible light so for this purpose UV analysis can be used for determination of adulteration in crude powder drugs.

Table 3: Fluorescence analysis of powdered of *Rhus succedanea* var. *himalaica* leaf and root.

S.NO	Drugs Reagents	Visible light	UV Low (250-270 nm)	UV High (360-390 nm)
1.	**Powder as such**	L. Br	Br	D.Br
2.	**50% HNO_3**	Gr	P	D. Gr
3.	**Picric acids**	Ye. G	Br	D. R
4.	**50% NH_3**	L. G	D. G	D. Gr
5.	**50% HCl**	Bl	D. P	D. R
6.	**H_2SO_4**	D. G	D.Br	D.R
7.	**NaOH**	G	D.G	D. Gr
8.	**Iodine**	G	Bl	D. Bl
9.	**$Fecl_3$**	Ye	D.Br	D. Gr
10.	**Methanol**	Gr	D. G	Bl
11.	**Diethyl ether**	Br	D. Bl	D. Br

Keys: Bl=Black, Br= Brown, Cr= Creamy, D= Dark, G=Green, Gr = Gray, L=Light, P= Pink, R= Red, Ye=Yellowish, Y= Yellow, W= White.

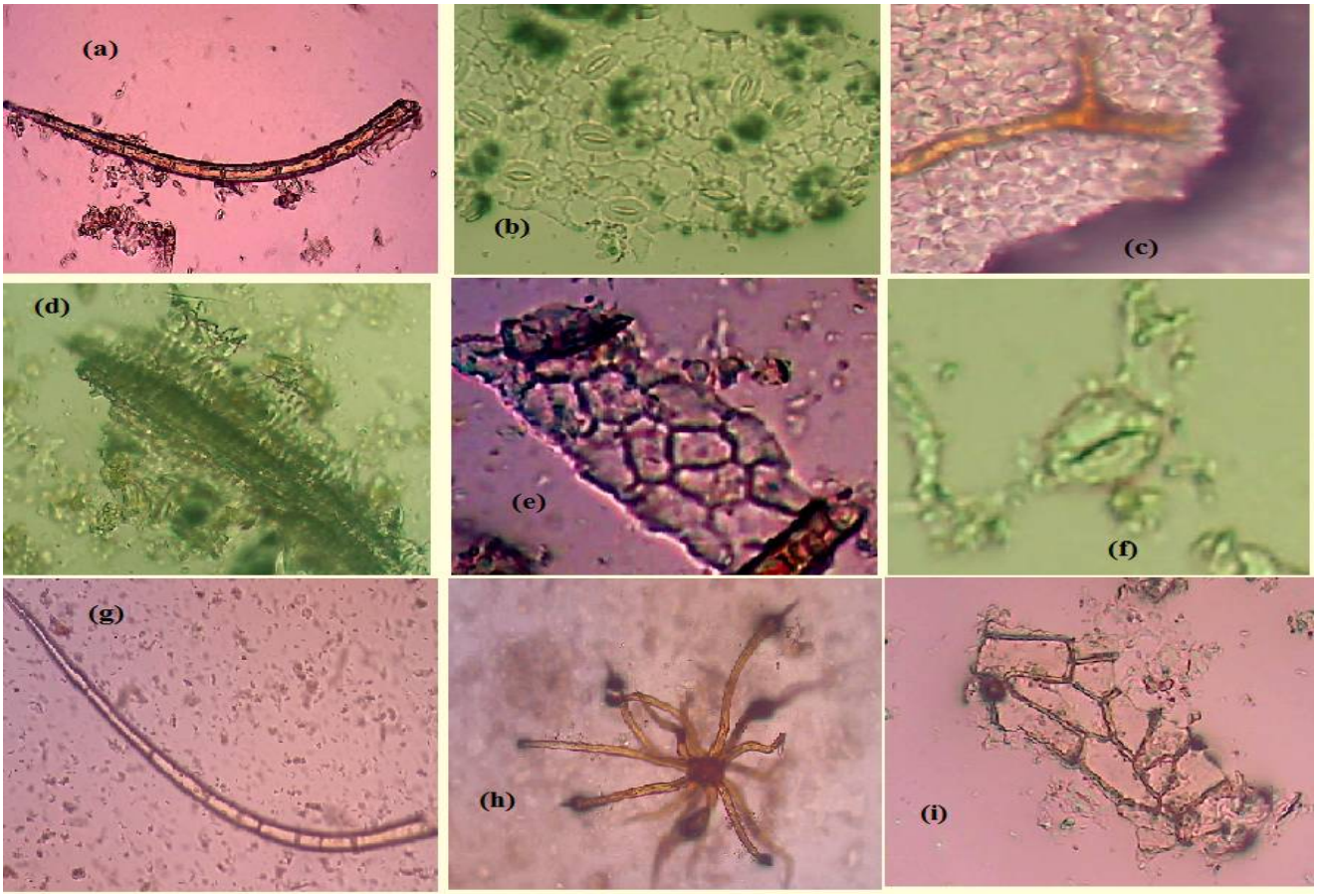
Extractive Values determination: In the present study the extractive value of leaf extract of *R. succedanea* was determined using various organic solvents like acetone, n-butane, methanol, ethanol chloroform and distilled water (Table. 4). The percent extractive values showed that leaf gives highest in ethanol (40.1%) followed by methanol (31.3%), chloroform (29.4%), acetone (26.3%), n-butane (22.2%) and the lowest in distilled water (15.2). The extractive values confirmed that the powder gives highest extraction in ethanol. Several investigators have carried out extractive values of various crude powder of plant using a number of organic and inorganic solvents, which strongly support the significance of this parameter in pharmacognostic evaluation, as e.g Khan & Khan (2013); Dinakaran et al. (2015); Zunjar et al. (2011) and Hussain et al. (2011) investigated the extractive values of *Crotalaria juncea*, *Rhazya stricta* and *Carica papaya* and *Hygrophila auriculata* K. Schum respectively. These workers concluded and suggested that extractive value determination is the main and cheap source of detection of adulterants, exhausted materials and selection of suitable solvent for extraction of crude powder in which it give highest amount of soluble constituents. In the current research the leaf of *R. succedanea* give highest amount of extractive values in ethanol. The present finding will be helpful for future phytochemical research on this plant.

Table 4: Percent extractive values of leaf and root of *R. Succedanea* with different solvents

Solvent Useds	Acetone	n-butane	Methanol	Ethanol	Chloroform	Distilled Water
Parts of Plant	26.3%	22.2%	31.3%	40.1%	29.2%	15.2%

Qualitative and Quantitative Phytochemical Screening: The therapeutic implication of natural plants is mainly dependent on the presence of active secondary phytoconstituents. Qualitative and quantitative phytochemical screening must be responsible for the detection of secondary metabolites in plants crude materials, having the pharmacological amplifications of the crude drugs and provides genuine drugs for companies and public health (Rai *et al*., (2013)). The leaf extract showed presence in large amount of carbohydrates, protein and amino acids, alkaloids, phenols, flavonoids and anthraquenones. Terpenoids were also detected while, saponins. Fixed oil and tannin showed complete absence (Table. 5). The amount and percent quantitative phtytochemical analysis of ethanolic crude extract of leaf for alkaloids, flavonoids and sterol showed highest amount of alkaloids (0.19mg/g) 19% followed by flavonoids (0.16mg/kg) 16% and sterols (0.15mg/kg) 15% (Table. 6; Figure 10).

Flavonoids showed the ability of altering immunological response and also have anti anaphylactic, anti-inflammatory, antioxidant, anti-allergic, antimicrobial and anticancer effects (Yun et al., 1996). Flavonoids have been reported to be used as antioxidant, analgesic, free radical scavenger and prevent the menopausal symptom in female (Antonisamy et al., (2012). The astringent properties of plants suggested was due to the presence of high amount of steroid and terpenoids, saponins and in relation with sex hormone and possessing strong analgesic effect. The tannins are used in bacterial, viral infections, burns, inflammation and wound healing (Savithramma et al (2011). The saponins and glycosides have to be used as immune-regulatory, anti-cancerous and in most of the cardiac diseases (Alamgir et al., (2014). Phenolic phytoconstituents documented to shows toxicity against pathogen, like bacteria and shows cytotoxic, anti-mutagenic, anti-oxidative and astringent properties (Edeoga et al., 2005). Anthraquinine metabolites are used as laxative, antimalarial and antineoplstic (Deore et al., (2015). Similar work was carried out by various investigators e.g, Yakubu & Salimon. (2015); Al-Snafi.(2015); Shilpashree et al. (2015); Soni & Sosa, (2013); Acharya et al. (2012); Singh, (2012); Hakemi et al.(2012); Majumdar, (2011) on sevrrak medicinal plants like *Mangifera indica, Chenopodium album, Catharanthus roseus, Peperomia pellucida, Ficus religiosa* respectively & suggest the importance of qualitative and quantitative phytochemical screening of crude drugs, which



greatly help the researchers in the field of phytochemistry and pharmacology to work advance research on medicinal plants in their respective field. The present work on *R. succedanea* will be of great help for further research on this plant in these fields.

Figure 9: Powder drug of *Rhus succednea var. himalaica* leaf
a- Non-glandular trichome, **b-** upper epidermis with anomocytic stomata, **c-** Lower epidermis having no stomata, **d-** helical to Spiral xylem vessels

Table 5: Qualitative screening tests of *Rhus succedanea var. himalaica* leaf and root
Keys: - = Not detected, + = Detected, ++= strongly Detected

S.NO	Constituents	Chemical tests	Leaf	Root
1.	Carbohydrates	Benedict Test	++	++
		Fehling Test	++	++
2.	Protein and amino acids	Ninhydrin Test	++	++
		Biuret Test	++	++
		Xanthoprotiec Test	+	+
3.	Alkaloids	Mayer,s Test	++	++
		Wagner,s Test	++	++
4.	Phenols	$FeCl_3$ Test	++	++
5.	Flavonoids	Alkaline reagent Test	+	+
		Lead acetate Test	++	++
6.	Fixed Oil		-	-
7.	Saponins	Frothing Test	-	+
		Foaming Test	-	+
		HCl Test	-	-
8.	Terpenoids	Salkowski,s Test	+	++
		Copper acetate Test	+	+
9.	Tannins	$FeCl_3$ Test	-	-
		Alkaline reagent Test	-	-
10.	Anthraquenones	Borntrager's Test	+++	+++

Table 6: Quantitative phytochemical analysis of leaf and root of *R. succedanea.* showing amount mg/g and percentage.

Phytochemicals		**Flavonoids**		**Alkaloids**		**Sterols**	
		Amount	**Percentage**	**Amount**	**Percentage**	**Amount**	**Percentage**
Parts of Plant	Leaf	0.16mg/g	16%	0.19mg/g	19%	0.15mg/g	15%
	Root	0.18 mg/g	18%	0.15mg/g	15%	0.11mg/g	11%

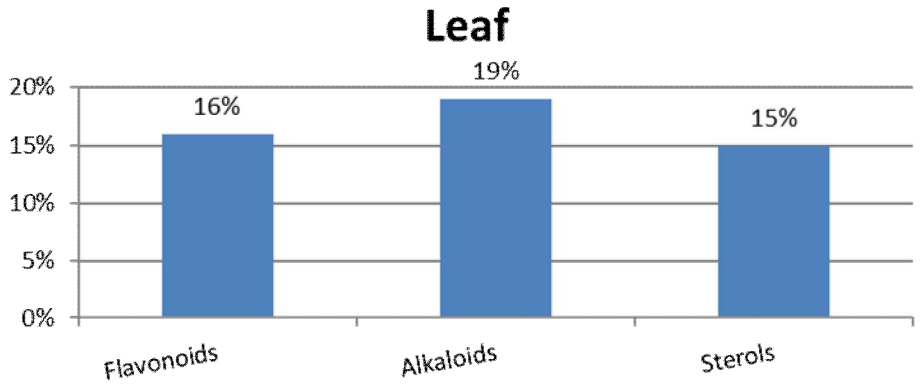


Figure 10: Quantitative percentage of phytochemical of leaf of *R. succedanea*

Conclusions and recommendation

R. succedanea belong to the family anacardiaceae, which is a perennial small sized deciduous tree; of about 5–9 m tall with opposite imparipinnately compound leaves, branched stem, small grayish yellow flower having paniculate Inflorescence and branched tape root showing secondary growth. The anatomical study of leaf showed numerous types of histological differentiation and the

numerical data observed was an important parameter for taxonomist in intra- specific difference. The organoleptic and physiochemical evaluation of leaf powder will be helpful in the standardization of this drug. The phytochemical screening showed the presence of carbohydrates, protein, alkaloids, phenols, flavonoids, terpenoids and anthraquenones, while the tannins and fixed oil was not detected. Quantitatively highest amount flavonoid (19%) in leaf was present which may be responsible for the strong pharmacological effects of the plant.

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