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Phytochemical Analysis of Selected Plant Leaves Consumed by the Folivory Bats in Tirunelveli

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Abstract

The study was designed to determine the phytochemical constituents of leaves consumed by the folivory bats, *Rousettus leschenaultia*, *Cynopterus sphinx* and *Pteropus giganteus*. Leaves are not only an important dietary source of minerals, carbohydrates, protein and calcium, it also contain secondary metabolites. The objectives of the study was to determine the chemical constituents of selected five plant species leaves of *Ficus religiosa*, *Erythrina indica*, *Coccinia grandis*, *Psidium guajava* and *Tamarindus indica*. Phytochemical analysis of the crude extract of the plant leaves revealed the presence of Phenols, Alkaloids, Flavinoids, Saponin, Tannin, Triterpenoids, Phytosteroid and Glycosides. The distribution of these phytochemical constituents of the leaves was assessed and compared. All the leaves were found to contain flavonoids and alkaloids. The presents of phytochemicals may have the major role in physical and biochemical activities of bats.

Keywords: Folivory, phytochemical, *Rousettus leschenaultia*, *Cynopterus sphinx* and *Pteropus giganteus.*

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Introduction

Bats are the only mammals that can fly. They are unique because of their capacity for flight and echolocation and their ability to hang upside down. Zoologists place the bats under the order known as Chiroptera. In Greek, the word chiro means hand, and ptera means wings. Their hands are modified to form a wing membrane which is a fold of skin stretched from the sides of the body to the elongated finger bones. The thumbs are free from the stretch of these wing membranes. The wings are divided into separate compartments by the elongated fingers. In this way, bats differ from the pterosaurs (the extinct flying reptile) whose wings were also folds of skin, but supported by a single elongated finger. The hind legs of bats also support the wing membranes. A few species of bats have a short or long tail which is either partly enclosed by the tail (interfemoral) membrane or extends between the two legs.

Bats are found in all parts of the world except the Arctic and Antarctic regions. The order Chiroptera comprising of nearly 850 species is the second largest in the world coming right after rodents (mice, squirrels, etc.). Regarding the type of food, the bats feed mainly on fruits like grapes, guavas, custard apples, bananas, papayas and chickoos. In addition they feed on leaves, petals and nectar.

Leaves are several plants Species are rich in protein (Telek and Martin, 1983; Kunz and Diaz 1995), they should provide an important sources of nitrogen for plant visiting bats (Kunz and Diaz 1995). Leaves are rich source of soluble nutrients of energy. This probably is the major factor in the evolution and obligate folivory in many terrestrial mammals. (Forman et al., 1979; Richardson et al., 1987). Bats that feed on leaves masticate them extract the liquid fraction along with nutrients and expel the chewed fibrous pellets (Kunz and Ingalls, 1994). Published reports of leaf consumption of bats were evidence that the liquid fractions of some leaves are high in protein. Telek and Martin (1983) and Lowry (1989) suggest that leaves provide an important source of dietary protein for fruit eating bats. Consumption of the Soluable fraction of protein rich leaves could provide the daily protein need of frugivorous bats (Kunz and Diaz, 1995). Because of the relatively low energy of leaves and potential energetic constraints that a diet high in fibre is likely to impose on flight, it is not surprising that true folivory (ingestion of whole leaves) is absent in bats (Kunz and Ingalls, 1994). The phytochemical analysis of the crude extract of the plant leaves eaten by bats were not yet studied. So the present work planned to investigate the presence of Phenols, Alkaloids, Flavinoids, Saponin, Tannin, Triterpenoids, Phytosteroid and Glycosides from the collected leaves. The distribution of these phytochemical constituents of the leaves were assessed and compared.

Materials and methods

The food items of three folivory bat species which are common in the study area are considered for the biochemical analysis. The Indian short-nosed fruit bat, *Cynopterus sphinx* is a frugivorous bat which belongs to the sub-order megachiroptera. It lacks echolocation but has a food sense of olfaction and vision. The characteristic features of this species are dog-shaped head, short ears with white margin and divergent opening of the nostrils. *C. sphinx* feeds upon fruits, leaves, floral parts and nectar

The fulvous fruit bat, *Rousettus leshenaulti* is a frugivorous bat `which belongs to the family pteropodidae and sub-order megachiroptera. The characteristic features of this species are the muscle is short and slender. The pelage is soft, fine and silky. It is fulvous brown on the crown of the head, back, flanks, and throat, the belly more grayish in the median area. The baculum is dumb-bell shaped. Proximal end is ovoid and larger than the distal. (Bates and Harrison, 1997)

The Indian flying fox, *Pteropus giganteus* is a very large bat with fore arm length of 168.4 mm and it belongs to sub-order megachiroptera. The characteristic features of this species are the snout is long and hairy throughout with the developed nostril. The ears are long and hairy throughout with well-developed nostrils. The ears are black, hairless, tall and pointed. The baculum is large and semicircular enclosing a heart shaped space. They feed on flowers and fruits, the solid discarded and soft pulps and juices swallowed. (Bates and Harrison, 1997)

The study was conducted on the campus of St. Johns college, in the town of Palayamkottai, South India (8^0 44'N,77^0 42'E), Where several tree species including *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamarindus indica were regularly visited by Rousettus leschenaultia, Cynopterus sphinx and Pteropus giganteus.* Samples were collected, weighed and placed in separate plastic storage bags.

Preparation of plant extracts

The shade dried plant material was powdered using kitchen blender and that powder was subjected to Soxhelet extraction with methanol (60^0C) for 24 hrs. Solvent extract was distilled and condensed at 40^0C. The condensed extract was stored at room temperature in air tight bottles and used for further studies.

Separation and Identification of phytochemicals of selected plants leaves

The presence of bioactive Phyto-compounds i.e. the secondary metabolites from the leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamarindus indica* were qualitatively analysed by thin layer chromatography.

TLC plate preparation

The solid phase of silica gel was kept in hot air oven at 100^0C for 20 minutes. Then the silica powder was mixed with petroleum ether and the slurry was prepared. The 20 x 20 cm clean TLC glass plates were taken and it was covered with that slurry and allowed to air dried. After drying the plates were kept in hot air oven in 72^0C for 1 hr. After developing the plates the condensed filtrate was spotted using capillary tube. The different spots were separated using a different solvent mixture act as mobile phase.

TLC study of alkaloids

About one gram powdered leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamarindus indica* were wetted with a half diluted NH_4OH and lixiviated with EtOAc for 24 hrs at RT. The organic phase is separated from the acidified filtrate and basified with NH_4OH (pH 11-12). It is extracted with chloroform (3x), condensed by evaporation and used for chromatography. The alkaloids spots were separated using the solvent mixture chloroform and methanol in the ratio of 15:1. The colour and hR_f value of the separated alkaloids were recorded both under Ultra Violet (UV 254 nm) and visible light after spraying with Dragendorff's reagent.

TLC study of flavonoids

The one gram powdered leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamarindus indica* were extracted with 10ml methanol on water bath (60^oC/5min). The filtrate was condensed by evaporation, added a mixture of water and EtOAc in the ratio of 10:1 and mixed thoroughly. The EtOAc phase thus retained is used for chromatography. The flavonoid spots were separated using chloroform and methanol solvent mixture in the ratio of 19:1. The colour and hRf value of these spots were recorded under Ultraviolet ($UV_{254nm)}$ light.

TLC study of glycosides

The powdered leaves of *Ficus religiosa, Erythrina indica, Cacinia grandis, Psidium guajava and Tamaridus indica* were extracted with 70% EtOH on rotary shaker (180 thaws/min) for 10hrs. 70% lead acetate was added to the filtrate and centrifuged at 5000rpm/min. Then the Supernatant was further centrifuged by adding 6.3% Na_2Co_3 at 10000 rpm/10min. The retained supernatant was dried, re-dissolved in chloroform and used for chromatography. The glycosides were separated using EtOAc-MeOH-H_2O solvent mixture in the ratio of 80:10:10. The colour and hRf value of these spots were recorded by observing under Ultraviolet ($UV_{254nm)}$ light.

TLC study of phenols

The powdered leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamarindus indica* were lixiviated in methanol on rotary shaker (180 thaws/min) for 24hrs. the condensed filtrate is used for chromatography. The phenols were separated using chloroform and methanol solvent mixture in the ratio of 27:0:3. The colour and hRf value of these spots were recorded by visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80^{o}C/10min.

TLC study of saponins

Two grams of powdered leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamaridus indica* were extracted with 10ml 70% EtOH by refluxing for 10 minutes. The filtrate was condensed, enriched with saturated n-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water solvent mixture in the ratio of 64:34:12:8. The colour and hRf value of these spots were recorded by exposing chromatogram to the iodine vapours.

TLC study of sterols

The powdered leaves of *Ficus religiosa, Erythrina indica, Coccinia grandis, Psidium guajava and Tamaridus indica* were extracted with 10ml methanol in water bath (80^{o}C/15min). The condensed filtrate is used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water solvent mixture in the ratio of 64:34:12:8. The colour and hRf value of these spots were recorded by visible light after spraying the plates with anaisaldeyde-sulphuric acid reagent and heating (100^{o}C/6 mins).

High performance liquid chromatography (HPLC)

HPLC analysis is carried out for the component separated in thin layer chromatography. It was performed on Shimadzu, Spintrom HPLC – 530 available in Science instrumentation Centre, CECRI, Karaikudi. The results were recorded.

Results and discussion

The result of Physical parameters of leaves were summarized in table 1, phytochemical screening of the plant leaves are summarized in table 2 and Chemical composition of organic components in leaves is summarized in table 3. Phytochemical analysis of food consumed by folivory bats was conducted in the present study secondary metabolites such as Phenols, Alkaloids, Flavonoids, Saponins, Tannin, Triterpenoides, Phytosterols, Carbohydrates and Glycosides were qualitatively and quantitatively analysed. (Table 1& 3).

Phytochemical analysis of sample reveals that all leaves contain Alkaloids, Flavonoids, and Carbohydrates. Three of them such as *Ficus religiosa, Psidium guajava and Tamarindus indica* contain Phenols. Saponins had present in *Ficus religiosa, Erythrina indica, and Tamarindus indica. Tannin* found in all the plant leaves except in *Erythrina indica. Three of them such as Ficus religiosa, Psidium guajava and Tamarindus indica* has contain Triterpenoides. Only *Erythrina indica,* and *Psidium guajava* has contain *phytosterols. Ficus religiosa, Erythrina indica and Coccinia grandis contained glycosides.*

Table1. Physical parameters of leaves investigated

S. No	Physical Parameters	*Ficus religiosa*	*Erythrina indica*	*Coccinia grandis*	*Psidium guajava*	*Tamaridus indica*
1	Colour	Dark green	Pale green	light green	Dark green	Pale green
2	Moisture	9.75%	11.5%	8.15%	17.35%	19.8%
3	Total Ash Content	46.5%	52.8%	34.25%	45.18%	51.12%
4	Ash Content (watersoluble)	21.05%	31.5%	11.05%	32%	24.8 %
5	Ash Content (Acid soluble)	11.25%	15 %	9.5%	8.5 %	7.5%
6	% of extract yield value in acetone	7.2%	9.1%	7.5%	11.3%	8%
7	% of extract yield value in ethanol	19.6%	21%	29%	41.5%	38.5%
8	% of extract yield value in chloroform	13%	19.8%	12.5%	18%	12%
9	% of extract yield value in benzene	8.5%	7%	8%	7.4%	6%
10	% of extract yield value in Methanol	24.5%	32.7%	45%	34%	37%
11	% of extract yield value in water	3.8%	4%	7%	5%	8%

Leaves are important to women plant visiting bats as their consumption may have stimulatory or inhibiting effects on reproductive activity. Two species of African fruit bats, *Epomorphorus wahlbergi* and *Rousettus aegyptiacus* are found to consume the leaves of *Balenties wilsoniana* (Wickler and Seibt, 1964). This plant contains two types of sapogenins *(Diosgenin and Yammosgenin)* which have been used in synthetic manufacture of steroid hormones (Sofoura and Hardman, 1973). Since steroids are essential for hormone synthesis, the oral intake of these compounds by the phytophagous bats may influence their reproductive activity (Wickler and seibt, 1964).

Secondary metabolites such as phenols may act as feeding deterrents and thus reduce the nutritional value of plant materials (Telek and martin, 1983). Compare with fruits, leaves are rich in protein (Milton, 1981). However, leaves also have structural and chemicals defense compounds that can defer potentional herbivores. Water content and toughness of leaves affective the extend of leaf damage by insert herbivores (Coley, 1983), whereas the levels of protein, fibre and condensed tannins and the protein-to-fibre ratio in leaves, influence the food choice of folivorous primates (Oates *et al*,1980; Mckey *et al.*,1981; Rogers *et al.*,1990; Kargupta and kumar,1994).

Table 2. Result of the phytochemical screening of the plant leaves investigated

S. No	Test	*Ficus religiosa*	*Erythrina indica*	*Coccinia grandis*	*Psidium guajava*	*Tamarindus indica*
1	Phenols Ferric chloride test	+	_	_	+	+
2	Alkaloids a. Mayer's test b.Dragendorff's test c. Wagner's test d. Hager's test	+ + + +	_ _ + +	+ + _ +	+ + + +	+ + + +
3	Flavonoids Shinoda test	+/-	+	+	+	+
4	Saponins Foam test	+	+	+	+	+
5	Tannin Lead acetate test	+	_	+	+	+
6	Triterpenoides Thionyl chloride Test	+	_	_	+	+
7	Phytosterols a. Liberman Burchard test b. Salkowski test	_ +	+ +	_ _	+ +	_ _
8	Carbohydrates a. Molish's test b. Reducing suger test	+ +	+ +	_ +	+ +	+ +
9	Glycosides a. Legal's test b. Borntrager's test c. Modified Borntrager's test	_ _ +/-	+ + +	_ + +	_ _ _	_ _ _ _

+ indicate present **- indicate absent**

Tannins represent an important group of plant secondary compounds that may adversely affect palatability and food intake (Oates *et al.*, 1977; 1980). Swain (1979) reported that a tannin content of 2.0% is enough to deter animals from eating some plant parts. These polyphenolic compounds, with a molecular weight between 500 and 20000, are able to from cross-linked complexes with proteins. These complexes are thought to be resistant to proteolysis at normal temperature in the digestive tract, and thus may reduce the availability of nitrogen to animals (Marquardt et al, 1978; Chibber *et al*, 1980). Notwithstanding, additional studies are needed to determine if secondary metabolites act as feeding deterrents, inhibitors of protein digestion, or as precursors to steroidal hormones and thus function as cues to reproduction (Kunz and Diaz,1995).

Given that alkaloids are related to herbivore resistance in a number of systems and that the near isogenic lines in this study were bred specifically to differ in alkaloids content, the most plausible explanation for reduced herbivory. This study is unique in that uptake of secondary compound was experimentally manipulated in living plants and uptake of these compounds influenced interactions with pollinators as well as herbivores (Lynn, 2000). Reiter (2003) analyzed the leaves consumed by *Pteropus jagori*, a frugivorous musky bat. He analysed the chemical composition of the leaves and stated that concentration of total phenol do not play a role in selection of leaves by *P.jagori* but the nutritional composition only plays a role in it.

Table. 3 Chemical compositions of organic components in leaves investigated

S.No.	Plant Name	Colour of the spot	Name of the Secondary metabolites Compound	R_f value
1.	*Ficus religiosa*	Pink	Alkaloids	74.59
		Yellow	Flavonoids	83.13
		Blue	Phenols	69.79
		Light yellow	Saponins	35.33
2.	*Erythrina indica*	Pink	Alkaloids	71.41
		Yellow	Flavonoids	84.53
			Phenols	-
		Light yellow	Saponins	43.83
3	*Coccinia grandis*	Pink	Alkaloids	64.46
		Yellow	Flavonoids	87.61
		Blue	Phenols	-
		Light yellow	Saponins	-
4.	*Psidium guajava*	Pink	Alkaloids	71.34
		Yellow	Flavonoids	73.52
		Blue	Phenols	89.12
		Light yellow	Saponins	-
5.	*Tamarindus indica*	Pink	Alkaloids	79.09
		Yellow	Flavonoids	86.30
		Blue	Phenols	89.03
		Light yellow	Saponins	45.03

Secondary metolotities are molecules that are not necessary for the growth and reproduction of a plant, but may steve some role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. Secondary compounds are often involved in key interactions between plants and their abiotic and environments that influence them (Facchini *et al.*, 2000). Phenols are also belonging to secondary metabolite category. Phenols, the aromatic compounds with hydrozy1 groups are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistance to bird attack. Phenols include an array of compounds like tannins, flavonols, etc. (Sadasivam and Manickam, 1996). We bio-chemically estimated quantitatively the amount of phenols present in fruits, leaves, flowers, and pollen consumed by frugivorous bats.

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