Toxicology International, *29(4) : 567-581; 2022. DOI: 10.18311/ti/2022/v29i4/30892*

INFORMATICS JOURNALS

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Phytochemical Characterization and Antioxidant Potential of Latex Extracts of Cardiotoxic Plants - *Cascabela thevetia* (L.) Lippold and *Plumeria alba* L.

Sanjukta Rajhans* and Himanshu Pandya

Department of Botany, Bioinformatics and Climate Change Impacts Management, School of Sciences, Gujarat University, Ahmedabad – 380009, Gujarat, India; sanjuktarajhans@gmail.com

ARTICLE INFO

KEYWORDS: Flavonoids, Latex, Phenols, Phytochemicals, DPPH

ARTICLE HISTORY

Received: 08.08.2022

Revised: 03.10.2022

Accepted: 06.10.2022

Abstract

The Apocynaceae family has been noted for their traditional and conventional medicinal properties for ages. The characteristic feature of this family is the milky sap or latex that is present in almost all the species. Many of the plants belonging to this family are a rich source of important drugs and are known for their pharmacological properties. Among the pharmacological properties, the antioxidant property is one that is known to protect against many chronic diseases including heart diseases and cancer. Two plants of the family i.e., *Cascabela thevetia* (L.) Lippold and *Plumeria alba* L. have been considered in the present experiment. Globally much research work has been conducted based on the different parts of these plants but, very little data is available on the latex portion. Hence in the present study, an attempt has been made to evaluate the phytochemical constituents present in the plants. Further, the total phenolic and flavonoid content and their correlation with the antioxidant potential have been evaluated.

1. Introduction

Apocynaceae is one of the largest families in the plant kingdom. Members of this family are rich in phytochemical compounds like glycosides, alkaloids, flavonoids, phenols, terpenes, etc. According to the updated classification by Endress *et al*., 2012 the family consists of 424 genera[1,2]. *Cascabela* and *Plumeria* are among them. With respect to its generic name *Thevetia*, the genus *Cascabela* has had a complicated history. According to the evaluation of the genus based on morphology-based phylogenetic analysis, it was suggested that *Cascabela* and *Thevetia* are sister taxa (Derived from the common ancestral node) and both of these should remain as separate genera[3]. Based on this information these taxa are recognized in two ways. For example, on one hand, a sequence of publications Morales 2009, Naz and Sulthana (2013), Alvarado-Cárdenas 2014, Gonzálex-Rocha and Cerros-Tlatilpa 2015 and the electronic databases The Plant List, (2013) acknowledged *Cascabela* as a separately accepted genus[4–7]. Whereas,

**Author for correspondence*

on the other hand, Endress *et al*., (2012), Williams and Stutzman (2008), Zarucchi (2009) subordinated the genus *Cascabela* under *Thevetia*[1,8,9]. In the present work, the genus is acknowledged as *Cascabela* and is according to The Plant List, (2013). *Cascabela thevetia* (L.) Lippold is cultivated throughout the tropics as an ornamental[6] and is known for its cardiotoxicity[10–13].

In the 17th century French botanist Charles Plumier, coined the name, Plumeria. But, before Plumier, it was Francisco de Mendoza, a Spanish priest who gave the name in the year 1522 Genus *Plumeria* belongs to the Apocynaceae family[14]. The plants of this genus are cultivated throughout the world and are found mostly in tropical and subtropical regions. *Plumeria* L. is native to tropical America and is found from southern Mexico to northern South America. In India, the plants of *Plumeria* L. are found almost abundantly. Most of the species are excellent ornamental plants, with attractive and fragrant flowers. *Plumeria alba* L. is a small shrub with a height of around 4.5 m. The plant is grown occasionally in the gardens for its ornamental and fragrant flowers. The plant is greatly known for its cardiotonic properties[15].

Both of these plants i.e., *Plumeria alba* L. and *Cascabela thevetia* (L.) Lippold is a laticiferous plant. Much research has been conducted globally using the different parts of these plants but, very few data are available on the latex portion of these plants. Hence, the phytochemical content and antioxidant potential of the latex extracts have been evaluated which would be of great use in future research works for developing medicinal drugs.

2. Materials and Methods

2.1 Latex Collection and Processing

The tender branches were used to extract fresh latex. New and sterile Eppendorf tubes were used for the collection of latex. The standard protocol of Aliyu *et al.,* (2015) was followed along with some modifications for preparing the latex extracts[16]. Here two solvents had been used i.e., water and methanol. Therefore, four samples were prepared in total. These samples were considered as the stock and were immediately stored at 0˚C to -18˚C for further use.

2.2 Preliminary Phytochemical Analysis

The preliminary phytochemical analysis is performed for evaluating the presence or absence of the phytochemicals in a particular extract. Hence, the plant material was subjected to preliminary phytochemical screening. The phytochemical profile of the latex extracts of both plants was determined according to the standard protocol of Banu and Cathrine (2015), Tiwari *et al*., (2011), and Velavan (2015)[17–19]. Table 1 represents preliminary phytochemical screening procedures with inferences.

Table 1. Preliminary phytochemical screening procedures with inferences

S. No.	PHYTOCHEMICAL	TEST	INFERENCE
1	Carbohydrates	Molisch's Test- 2ml of extract+ 4 drops of alcoholic solution of α-naphthol+ 2 drops of conc. Sulphuric acid added slowly along the sides of a test tube	The development of a violet ring indicates the presence of carbohydrates
		Benedict's Test- Extracts were treated with Benedict's reagent and heated gently	Orange-red precipitates indicate the presence of carbohydrates
2	Proteins	Millon's Test- 2ml of extract + a few drops of Millon's reagent	White precipitates indicate the presence of proteins
		Biuret Test- 2ml of extract + 2 drops of 2% copper sulphate solution + 1ml of ethanol + Potassium hydroxide pellets	The pink color ethanolic layer indicates the presence of proteins
3	Alkaloids	Dragendroff's Test- 1 ml of extract + 1 ml Dragendroff's reagent	Red precipitates indicate the presence of alkaloids

		Hager's Test- 1ml of extract + 1 ml of Hager's reagent	Yellow precipitates indicate the presence of alkaloids
4	Glycosides	Keller Killiani Test-1 ml of extract + 5 ml of water + 1 drop of Glacial acetic acid + 1 drop of ferric chloride solution + 1 drop of conc. Sulphuric acid	A Brown ring indicates the presence of cardenolides. A Violet ring below a brown ring or green ring below a brown ring indicates the presence of glycosides
		Legal's Test-1 ml of extract + pyridine + sodium nitroprusside solution + 10% NaOH	Pink to blood red color indicates the presence of glycosides
5	Phenolic Compounds and Tannins	Ferric Chloride Test- 1 ml of extract + 5 ml of distilled water + 3 drops of 5% ferric chloride solution	The dark green color indicates the presence of phenolic compounds
		Lead Acetate Test- 1 ml of extract + 3 ml of 10% lead acetate solution	A bulky white precipitate indicates the presence of phenolic compounds and tannins
6	Terpenoids	Liberman Burchard Test- 1 ml of extract + 2 ml acetic anhydride + 1-2 drops of conc. Sulphuric acid	An array of color changes indicates the presence of terpenoids
	Diterpenes	Copper acetate Test- 1ml of extract + 3-4 drops of copper acetate solution	The Emerald green color indicates the presence of diterpenes
7	Flavonoids	Alkaline Reagent Test- 1 ml of extract + few drops of sodium hydroxide solution	The formation of intense yellow color indicates the presence of flavonoids
		Shinoda Test- 2ml of extract + 50% methanol + Heat + magnesium metal + a few drops of conc. HCl	Green to blue coloration indicates the presence of flavonoids
8	Steroids	Salkowski Test- 1 ml of extract + 3 ml of chloroform + 3 drops of conc. Sulphuric acid is shaken and allowed to stand	The appearance of golden yellow color indicates the presence of steroids
		Liberman Burchard Test- 1 ml of extract + 1 ml chloroform + 3 few drops acetic anhydride boiled and cooled + conc. Sulphuric acid	The brown ring at the junction indicates the presence of steroids
9	Saponins	Foam Test- 1ml of extract + 2ml of water with continuous shaking	The foam produced and persists, indicates the presence of saponins
		Froth Test- 1ml of extract + 5ml of distilled water with vigorous shaking + 3 drops of olive oil	The formation of emulsion indicates the presence of saponins
10	Coumarins	Test for Coumarins- 1ml of extract+ 10% Sodium hydroxide	Blue-green fluorescence indicates the presence of coumarins
11	Gums and mucilage	Test for Gums and Mucilage- 1ml of extract + 2ml of absolute alcohol with constant stirring	Cloudy precipitate indicates the presence of gums and mucilage
12	Oils and Fats	Spot Test- Small quantity of extracts pressed between two filter papers	Oil stain indicates the presence of oils and fats

2.3 FTIR Analysis

FTIR is a technique used for acquiring the infrared spectrum of absorption or emission of a solid, liquid, or gas. This technique can be used to assemble high spectral resolution data over a wide range of wavelengths[20].

The FTIR study of latex extracts was carried out using the FTIR instrument (Bruker- ALPHA). The extracts were kept on the sample holder and were scanned from the spectral region of 4000 to 400 cm^{-1}. The observed FTIR spectra were used for the detection of various functional groups present in the samples.

2.4 Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocâlteu method along with some modifications[21]. A stock solution of 1mg/ml of gallic acid had been prepared. Series was as follows- 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and100µg/ml. To this 500 µl of Folin-Ciocâlteu reagent and 400 µl of 7% of Sodium carbonate (Na_2CO_3) were added. Further, the total volume was made up to 2000µl. An incubation period of 30 min was provided before taking the O. D. at 765 nm in a UV-Visible Spectrophotometer. The same procedure was repeated for the latex extracts. The total phenolic content of the samples was expressed in µg of gallic acid equivalents (GAE)/ml of latex.

2.5 Total Flavonoid Content

Total flavonoid content was determined using the protocol of Saini and Mishra 2018, and Dubey *et al*., 2020 along with some modifications[21,22]. A stock solution of 1mg/ml of quercetin had been prepared. Series was as follows- 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and100µg/ml. To this 10 µl of Aluminum chloride ($AlCl_3$) and 10 µl of 7% of Sodium acetate ($C2H_3NaO_2$), were added. Further, the total volume was made up to 2000µl. An incubation period of 45 min was provided before taking the O. D. at 415 nm in a UV-Visible Spectrophotometer. The same procedure was repeated for the latex extracts and all the procedures were done in triplicates. The total flavonoid content of the samples was expressed in µg of quercetin equivalents (QE)/ml of latex.

2.6 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is used for screening the antioxidant activity of the plant extracts. The free radical scavenging activity of the latex extracts was determined by the DPPH assay. The protocol of Mata *et al*., (2015) was followed along with some modifications[23]. The latex extracts were taken in series from the stock solutions (1mg/ml) and to this, the respective solvents were added along with 2 ml of 0.1mM DPPH. The total volume was made up to 3 ml. The mixture was incubated in dark for 30 minutes, at 37˚C. After the time period of 30 minutes, the absorbance was measured using a spectrophotometer (Shimadzu UV-1800) at 517nm. In this study, L-Ascorbic Acid was considered the standard. In the blank only the solvents were considered respectively whereas, 1 ml of solvent with 2 ml of DPPH was considered as control. The experiments were performed in triplicates. % Inhibition was calculated using the following formula

% inhibition = [(Absorbance of control-Absorbance of sample) x 100/Absorbance of control]

3. Results

3.1 Preliminary Phytochemical Analysis

Results of the preliminary phytochemical tests of the plant latex extracts has been presented in Table 2. The aqueous latex extract of *Cascabela thevetia* (L.) Lippold (CTA) showed the presence of carbohydrates, proteins, glycosides, phenolic compounds, tannins, terpenoids, diterpenes, flavonoids, steroids, saponins, coumarins, gums, and mucilage. Alkaloids, oils and fats were found to be completely absent. The methanolic latex extract of *Cascabela thevetia* (L.) Lippold (CTM) showed the presence of carbohydrates, proteins, glycosides, phenolic compounds, tannins, terpenoids, diterpenes, flavonoids, steroids, coumarins, gums, mucilage, oils, and fats. Whereas, alkaloids and saponins were found absent in the CTM extract.

In the aqueous latex extract of *Plumeria alba* L. (PAA) carbohydrates, proteins, glycosides, phenolic compounds, tannins, terpenoids, diterpenes, flavonoids, steroids, saponins, coumarins, gums, mucilage, oils, and fats were found present. Whereas, the PAA extract was devoid of alkaloids. In the methanolic latex extract of *Plumeria alba* L. (PAM) proteins, glycosides, phenolic compounds, tannins, flavonoids, steroids, saponins, gums and mucilage were found present. While, the PAM extract was devoid of carbohydrates, alkaloids, terpenoids, diterpenes, coumarins, oils, and fats.

Table 2. Preliminary phytochemical tests of the plant latex extracts

S.No.	Name of the Main Phytochemical Compound	Name of the Test	CTA	CTM	PAA	PAM
1	Carbohydrates	Molisch's Test	+++	++	+++	-
		Benedict's Test	+	+	+	-
2	Proteins	Millon's Test	+	+++	+	+++
		Biuret Test	+	+++	+	+++
3	Alkaloids	Dragendroff's Test	-	-	-	-
		Hager's Test	-	-	-	-
4	Glycosides	Keller Killiani Test	+++	+++	+	++
		Legal's Test	++	+++	+	+
5	Phenolic Compounds and Tannins	Ferric Chloride Test	++	+++	+	++
		Lead Acetate Test	+	++	+	++
6	Terpenoids	Liberman Burchard Test	+	+++	+	-
	Diterpenes	Test for Diterpenes	++	+++	+	-
7	Flavonoids	Alkaline Reagent Test	++	++	+	++
		Shinoda Test	+	++	+	++
8	Steroids	Salkowski Test	+	+++	++	+++
		Liberman Burchard Test	+	++	+	+
9	Saponins	Foam Test	+	-	+	+
		Froth Test	+	-	+	+
10	Coumarins	Test for Coumarins	+	+++	+	-
11	Gums and mucilage	Test for Gums and Mucilage	+	+++	+	+++
12	Oils and Fats	Spot Test	-	++	++	-

(Where [-] = Absent, [+] = Low Concentration, [++] = Medium Concentration and [+++] = High Concentration; CTA= aqueous latex extract of *Cascabelathevetia*(L.) Lippold, CTM= methanolic latex extract of *Cascabelathevetia*(L.) Lippold, PAA= aqueous latex extract of *Plumeria alba*L., PAM= methanolic latex extract of *Plumeria alba* L.)

It was observed that alkaloids were absent in all the extracts. In the methanolic latex extracts of both plants, it was observed that proteins, gums, and mucilage were present in higher concentrations as compared to other phytochemicals.

3.2 FTIR Analysis

FTIR is an analytical instrument used for analyzing organic and inorganic samples. It is used for detecting functional groups. Here, in the current study FTIR analysis of all four

extracts was conducted. The results have been presented as follows

3.2.1 *Cascabela thevetia* (L.) Lippold Aqueous Latex Extract (CTA)

The results obtained from Figure 1 (a) and Table 3 exhibit the presence of carboxylic acid with a strong peak at 3264.1943 cm^{-1} representing the O-H stretching functional group. Medium peaks were obtained at 1635.7743 cm^{-1}, 573.5405 cm^{-1}, 559.1687 cm^{-1}, 543.4511 cm^{-1}, 520.2624 cm^{-1} exhibiting N-H bending and C-Br stretching functional groups. This further confirms to the presence of amines and alkyl halides. Small peaks were obtained at 623.1177 cm^{-1} and 607.6909 cm^{-1} with functional group C=C-H: C-H bending showing the presence of alkynes. Weak peaks were also observed at 2199.7859 cm^{-1}, 2110.6953 cm^{-1}, 1992.1044 cm^{-1}, and 1964.1247 cm^{-1} alkyne and an aromatic class of compounds were found present.

3.2.2 *Cascabela thevetia* (L.) Lippold Methanolic Latex Extract (CTM)

The results obtained from Figure 1 (b) and Table 4 clearly exhibit the presence of carboxylic acid, alcohols, esters and ethers with a strong peak at 1014.9270 cm^{-1} representing the C-O stretching functional group. Medium peaks were obtained at 3317.6462 cm^{-1}, 1647.2539 cm^{-1}, 1450.2435 cm^{-1}, 1413.9220 cm^{-1}, 1244.6896 cm^{-1}, 1113.0569 cm^{-1}, 552.3479 cm^{-1}, 535.4771 cm^{-1} and 520.8303 cm^{-1} exhibiting N-H stretching, C=N stretching, C-H bending, O-H bending, C-N stretching, C-Br stretching functional groups. This further confirms the presence of aliphatic primary amine, imine/oxime, alkane, alcohol, amine, and alkyl halides.

Table 3. FTIR interpretation of compounds of *Cascabela thevetia* (L.) Lippold aqueous latex extract

S.No (CTA)	Wave Number (cm^{-1})	Frequency Range	Appearance	Functional Group	Compound Class
1	3264.1943	4000-3000 cm^{-1}	Strong	O-H Stretching	Carboxylic acid
2	2199.7859	2400-2000 cm^{-1}	Weak	C≡C Stretching	Alkyne
3	2110.6953	2400-2000 cm^{-1}	Weak	C≡C Stretching	Alkyne
4	1992.1044	2000-1650 cm^{-1}	Weak	C-H Bending	Aromatic compound
5	1964.1247	2000-1650 cm^{-1}	Weak	C-H Bending	Aromatic compound
6	1635.7743	1670-1600 cm^{-1}	Medium	N-H Bending	Amine
7	623.1177	1000-650 cm^{-1}	Small	C=C-H: C-H Bending	Alkynes
8	607.6909	1000-650 cm^{-1}	Small	C=C-H: C-H Bending	Alkynes
9	573.5405	1000-650 cm^{-1}	Medium	C-Br Stretching	Alkyl halides
10	559.1687	1000-650 cm^{-1}	Medium	C-Br Stretching	Alkyl halides
11	543.4511	1000-650 cm^{-1}	Medium	C-Br Stretching	Alkyl halides
12	520.2624	1000-650 cm^{-1}	Medium	C-Br Stretching	Alkyl halides

Table 4. FTIR interpretation of compounds of *Cascabela thevetia* (L.) Lippold methanolic latex

S.No (CTM)	Wave Number (cm^{-1})	Frequency Range	Appearance	Functional Group	Compound Class
1	3317.6462	4000-3000 cm-1	Medium	N-H stretching	Aliphatic primary amine
2	2946.8947	4000-3000 cm-1	Weak	O-H Stretching	Alcohol
3	2835.3011	4000-3000 cm-1	Weak	O-H Stretching	Alcohol
4	2200.4542	2400-2000 cm-1	Weak	C≡C Stretching	Alkyne
5	2111.5909	2400-2000 cm-1	Weak	C≡C Stretching	Alkyne
6	1992.1984	2000-1650 cm-1	Weak	C-H Bending	Aromatic compound
7	1964.9921	2000-1650 cm-1	Weak	C-H Bending	Aromatic compound
8	1647.2539	2000-1650 cm-1	Medium	C=N Stretching	Imine/Oxime
9	1450.2435	1600-1300 cm-1	Medium	C-H Bending	Alkane
10	1413.9220	1400-1000 cm-1	Medium	O-H Bending	Alcohol
11	1244.6896	1400-1000 cm-1	Medium	C-N Stretching	Amine
12	1113.0569	1400-1000 cm-1	Medium	C-N Stretching	Amine
13	1014.9270	1400-1000 cm-1	Strong	C-O Stretching	Alcohols, Carboxylic acids, Esters, Ethers
14	678.3881	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
15	657.1280	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
16	638.2338	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
17	552.3479	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides
18	535.4771	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides
19	520.8303	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides

Small peaks were obtained at 678.3881 cm^{-1}, 657.1280 cm^{-1}, and 638.2338 cm^{-1} with functional group C=C-H: C-H bending showing the presence of alkynes. Weak peaks were also observed at 2946.8947 cm^{-1}, 2835.3011 cm^{-1},

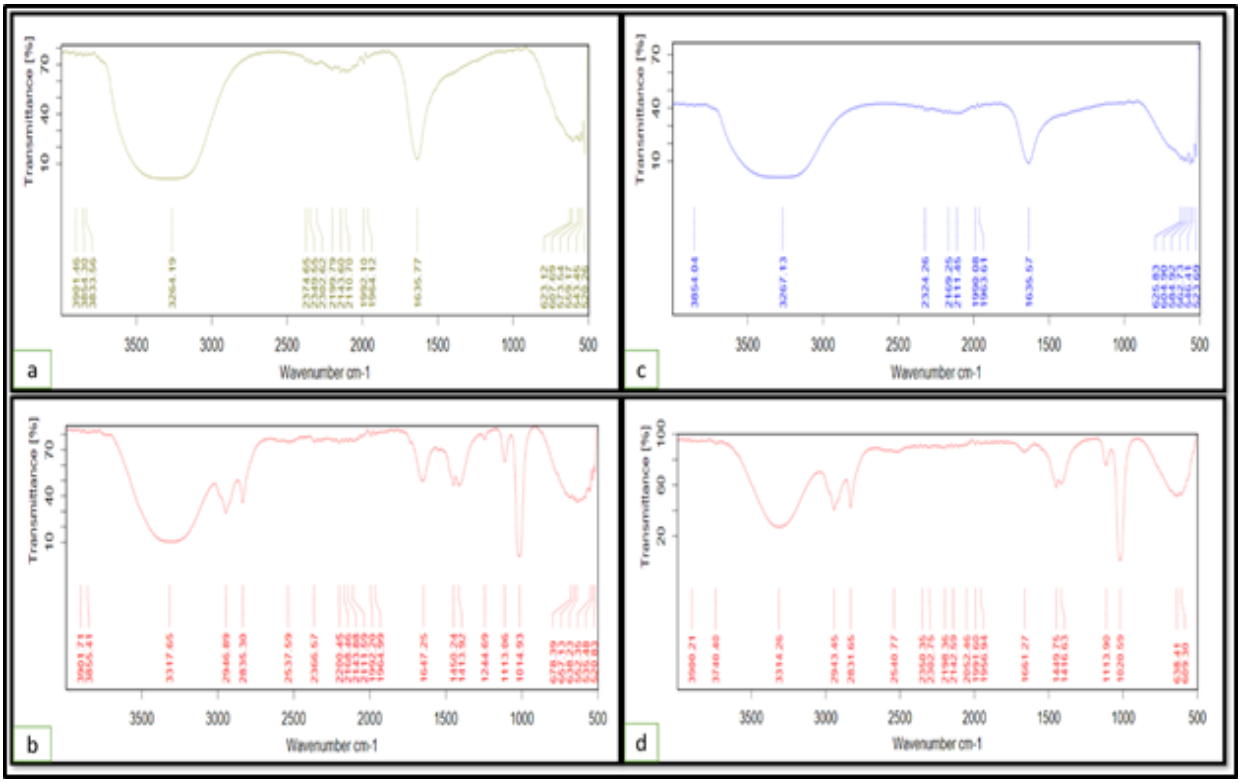


Figure 1. FTIR analysis of **(a)** *Cascabela thevetia* (L.) Lippold aqueous latex extract. **(b)** *Cascabela thevetia* (L.) Lippold methanolic latex extract. **(c)** *Plumeria alba* L. aqueous latex extract. **(d)** *Plumeria alba* L. methanolic latex extract.

2200.4542 cm^{-1}, 1992.1984 cm^{-1}, 1964.9921 cm^{-1} alcohol, alkyne, and an aromatic class of compounds were found present.

3.2.3 *Plumeria alba* L. Aqueous Latex Extract (PAA)

The results obtained from Figure 1 (c) and Table 5 exhibit the presence of carboxylic acid with a strong peak at 3267.1340 cm^{-1} representing the O-H stretching functional group. Medium peaks were obtained at 1635.5732 cm^{-1}, 584.9162 cm^{-1}, 562.7350 cm^{-1}, 546.4132 cm^{-1}, and 523.6883 cm^{-1} exhibiting N-H bending and C-Br stretching functional groups. This further confirms the presence of amines and alkyl halides. Small peaks were obtained at 625.8323 cm^{-1} and 604.8966 cm^{-1} with functional group C=C-H: C-H bending showing the presence of alkynes. Weak peaks were also observed at 2111.4456 cm^{-1}, 1990.0820 cm^{-1}, 1963.6061 cm^{-1} alkynes, and an aromatic class of compounds were found present.

3.2.4 *Plumeria alba* L. Methanolic Latex Extract (PAM)

The results obtained from Figure 1 (d) and Table 6 exhibit the presence of carboxylic acids, alcohols, esters, and ethers with a strong peak at 1020.5927 cm^{-1} representing the C-O stretching functional group. Medium peaks were obtained at 3314.2612 cm^{-1}, 1661.2699 cm^{-1}, 1449.7456 cm^{-1}, 1416.6253 cm^{-1}, 1113.9031 cm^{-1} exhibiting N-H stretching, C=C stretching, C-H bending, O-H bending, C-N stretching functional groups. This further confirms the presence of aliphatic primary amine, alkene, alkane, alcohol, and amine classes of compounds. Small peaks were obtained at 638.4107 cm^{-1}, 609.3002 cm^{-1} with functional group C=C-H: C-H Bending showing the presence of alkynes. Weak peaks were also observed at 2943.4517 cm^{-1}, 2831.6508 cm^{-1}, 2198.3630 cm^{-1}, 1991.5993 cm^{-1}, 1956.9351 cm^{-1} alcohol, alkyne, and an aromatic class of compounds were found present.

Table 5. FTIR Interpretation of compounds of *Plumeria alba* L. aqueous latex extract

S.No (PAA)	Wave Number (cm^{-1})	Frequency Range	Appearance	Functional Group	Compound Class
1	3267.1340	4000-3000 cm-1	Strong	O-H Stretching	Carboxylic acid
2	2111.4456	2400-2000 cm-1	Weak	C≡C Stretching	Alkyne
3	1990.0820	2000-1650 cm-1	Weak	C-H Bending	Aromatic Compound
4	1963.6061	2000-1650 cm-1	Weak	C-H Bending	Aromatic Compound
5	1635.5732	1670-1600 cm-1	Medium	N-H Bending	Amine
6	625.8323	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
7	604.8966	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
8	584.9162	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides
9	562.7350	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides
10	546.4132	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides
11	523.6883	1000-650 cm-1	Medium	C-Br Stretching	Alkyl halides

Table 6. FTIR interpretation of compounds of *Plumeria alba* L. methanolic latex extract

S.No (PAM)	Wave Number (cm^{-1})	Frequency range	Appearance	Functional Group	Compound Class
1	3314.2612	4000-3000 cm-1	Medium	N-H stretching	Aliphatic primary amine
2	2943.4517	4000-3000 cm-1	Weak	O-H stretching	Alcohol
3	2831.6508	4000-3000 cm-1	Weak	O-H stretching	Alcohol
4	2198.3630	2400-2000 cm-1	Weak	C≡C stretching	Alkyne
5	1991.5993	2000-1650 cm-1	Weak	C-H bending	Aromatic compound
6	1956.9351	2000-1650 cm-1	Weak	C-H bending	Aromatic compound
7	1661.2699	1670-1600 cm-1	Medium	C=C stretching	Alkene

8	1449.7456	1600-1300 cm-1	Medium	C-H bending	Alkane
9	1416.6253	1400-1000 cm-1	Medium	O-H bending	Alcohol
10	1113.9031	1400-1000 cm-1	Medium	C-N Stretching	Amine
11	1020.5927	1400-1000 cm-1	Strong	C-O Stretching	Alcohols, Carboxylic acids, Esters, Ethers
12	638.4107	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes
13	609.3002	1000-650 cm-1	Small	C=C-H: C-H Bending	Alkynes

3.2.5 Total Phenolic Content

The total phenolic content of the four latex extracts was expressed as µg of gallic acid equivalents (GAE)/ml of latex using the equation obtained from the standard curve Figure 2 (a). The equation for the aqueous extracts obtained was $y = 0.0251x + 1.4736$, $R^2 = 0.999$ and for the methanolic extracts was $y = 0.0231x + 1.7558$, $R^2 = 0.9933$. Here, x is the absorbance at 760 nm and y is the total phenolic content in the latex extracts. Here, R^2 is the coefficient value. Table 7 and Figure 2 (c) represent the total phenolic content at the particular concentration. It was observed that with the increase in the concentration the TPC also increased. The highest TPC was observed in the CTM extract followed by PAM, CTA, and PAA. Hence, the order of TPC observed was CTM> PAM> CTA> PAA.

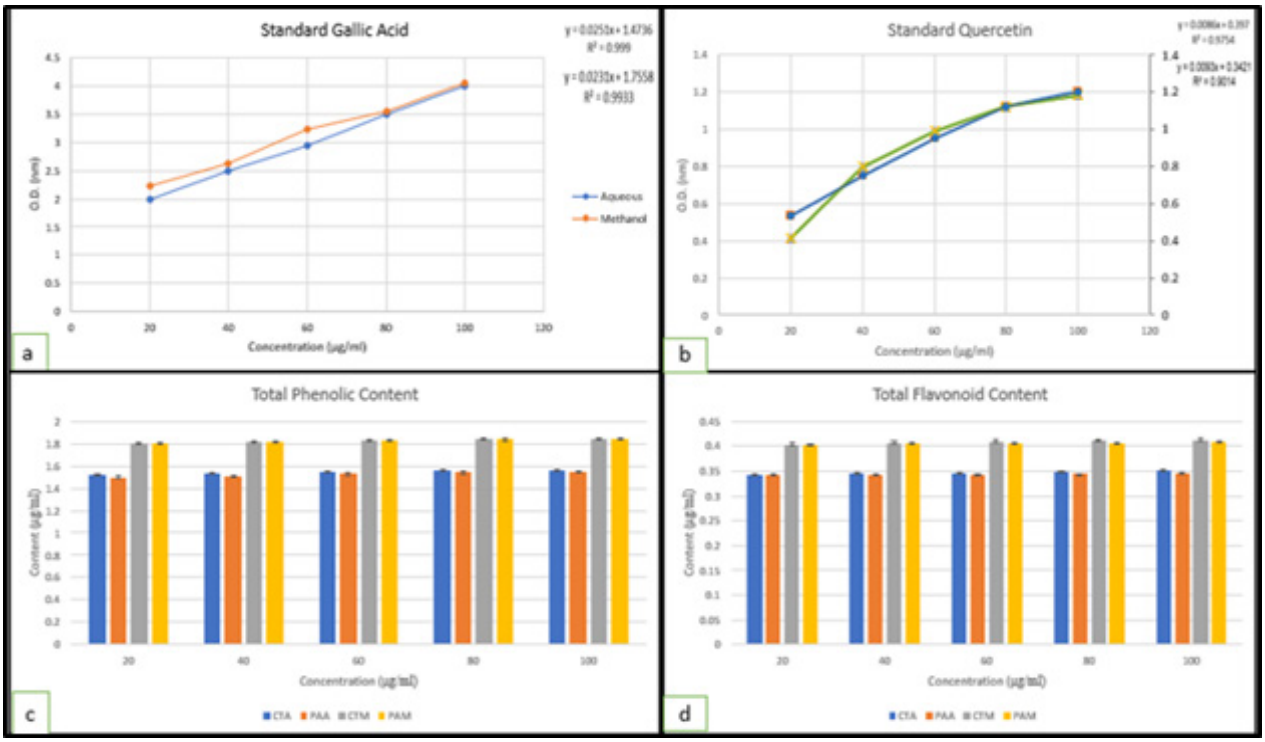


Figure 2. **(a)** Calibration curve of Gallic Acid (Standard). **(b)** Calibration curve of Quercetin (Standard). **(c)** Total Phenolic Content of the plant latex extracts. **(d)** Total Flavonoid Content of the plant latex extracts.

Table 7. Total Phenolic Content in different latex extracts of the plants *Cascabela thevetia* (L.) Lippold and *Plumeria alba* L.

Concentration (µg/ml)	TPC Content			
	CTA	PAA	CTM	PAM
20	1.526 ± 0	1.499 ± 0	1.808 ± 0.011	1.802 ± 0
40	1.535 ± 0.057	1.511 ± 0	1.821 ± 0.017	1.815 ± 0.011
60	1.551 ± 0	1.531 ± 0.005	1.829 ± 0.005	1.824 ± 0.011
80	1.560 ± 0.005	1.543 ± 0.005	1.837 ± 0.011	1.836 ± 0.017
100	1.563 ± 0	1.549 ± 0.011	1.848 ± 0	± 0.011

3.2.6 Total Flavonoid Content

The total flavonoid content of the four latex extracts was expressed asµg of quercetin equivalents (QE)/ ml of latex using the equation obtained from the standard curve Figure 2 (b). The equation for the aqueous extracts obtained was y = 0.0093x + 0.3421, R^2 = 0.9014 and for the methanolic extracts was y = 0.0086x + 0.397, R^2 = 0.9754. Here, x is the absorbance at 415 nm and y is the total flavonoid content in the latex extracts. Here, R^2 is the coefficient value. Table 8 and Figure 2 (d) represent the total flavonoid content at the particular concentration. It was observed that with the

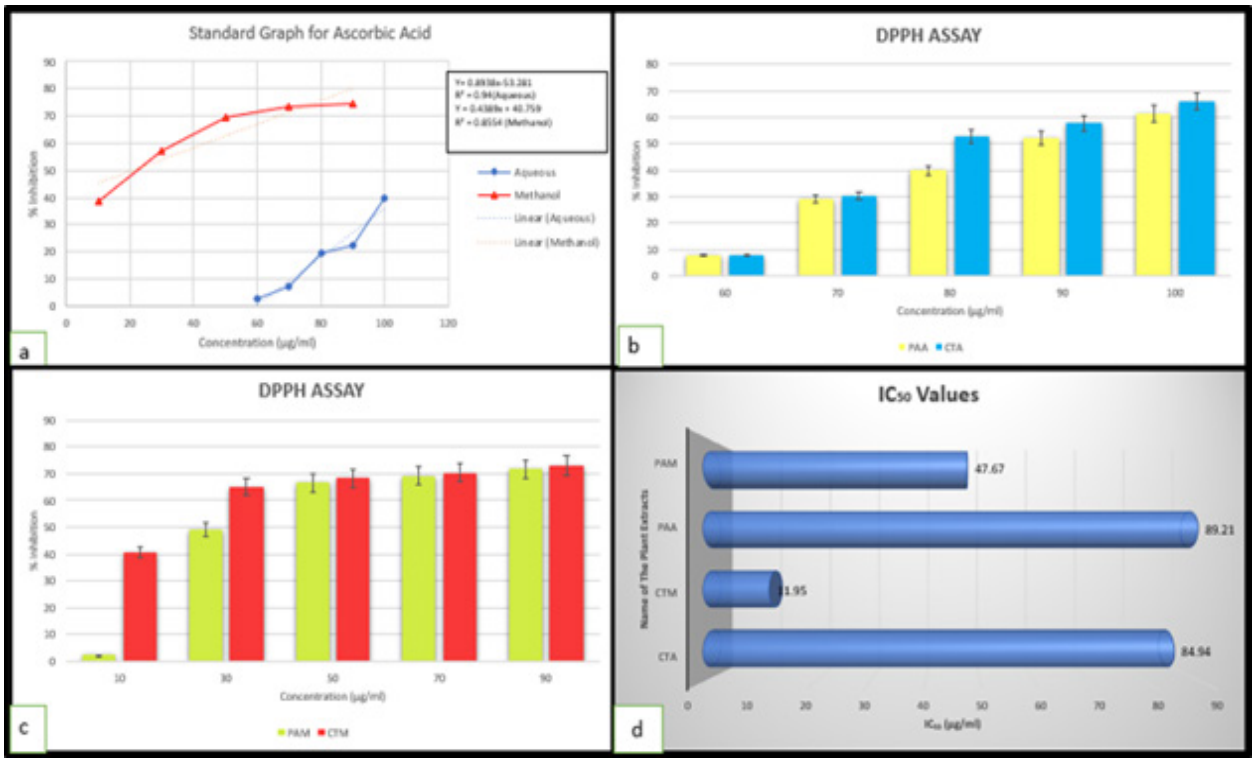


Figure 3. **(a)** Standard graph for Ascorbic acid for DPPH assay. **(b)** The % inhibition of DPPH assay of different plant aqueous latex extracts [where PAA= Aqueous latex extract of *Plumeria alba* L. and CTA= Aqueous latex extract of *Cascabela thevetia* (L.) Lippold]. **(c)** The % inhibition of DPPH assay of different plant methanolic latex extracts [where PAM= Methanolic latex extract of *Plumeria alba* L. and CTM= Aqueous latex extract of *Cascabela thevetia* (L.) Lippold]. **(d)** Graphical representation of IC_{50} values of DPPH assay of the plant latex extracts

Table 8. Total Flavonoid Content in different latex extracts of the plants *Cascabela thevetia* (L.) Lippold and *Plumeria alba* L.

Concentration (µg/ml)	TFC Content			
	CTA	PAA	CTM	PAM
20	0.343 ± 0	0.342 ± 0.057	0.404 ± 0.005	0.403 ± 0.011
40	0.344 ± 0	0.343 ± 0	0.407± 0.011	0.405± 0
60	0.345 ± 0.005	0.3438 ± 0.005	0.410 ± 0.017	0.406 ± 0
80	0.349 ± 0.005	0.344 ± 0	0.411± 0.011	0.407 ± 0
100	0.351 ± 0	0.345± 0.011	0.413± 0.005	± 0.243

Table 9. Concentration and percentage inhibition with standard error for DPPH assay

Extracts	Concentrations (µg/ml)	% Inhibition
CTA	60, 70, 80, 90, 100	7.731±0.005, 30.41±0.041, 52.57±0.001, 57.73±0.005, 65.97±0.0003
PAA	60, 70, 80, 90, 100	7.6923±0.002, 28.84±0.004, 39.74±0.004, 51.92±0.009, 61.53±0.008
CTM	10, 30, 50, 70, 90	40.43±,0.003 64.98±0.003, 68.23±0.002, 70.39±0.0003, 72.92±0.002
PAM	10, 30, 50, 70, 90	2.33±0.17, 49.33±0.05, 66.66±0.003, 69.33±0.001, 71.67±0

increase in the concentration the TFC also increased. The highest TFC was observed in the CTM extract followed by PAM, CTA, and PAA. Hence, the order of TPC observed was CTM> PAM> CTA> PAA.

3.2.7 DPPH Assay

The free radical scavenging ability of the plant latex extracts was determined by the DPPH assay. DPPH produces a purple-to-violet colour in solution which in the presence of antioxidants gets fades into yellow colour. Here the DPPH assay was conducted to determine if any antioxidant potential is present in plant latex extracts. In cancer and cardiovascular diseases, antioxidant properties play an important role. Hence, the DPPH assay was conducted for finding out the antioxidant potential of the latex extracts.

The DPPH assay was performed for all four latex extracts. Ascorbic acid was used as the standard. The calibration curve was plotted using the ascorbic acid as shown in Figure 3 (a) below.

Table 9 and Figures 3 (a) and 3 (b) represent the concentrations and % inhibition of all the latex extracts. Figure 3 (b) represents the % inhibition of aqueous latex extracts and Figure 3 (c) represents the % inhibition of methanolic latex extracts.

Based on the above Table 9 and Figure 3 (b) it was observed that the % inhibition of the CTA (aqueous latex extract of *Cascabela thevetia* (L.) Lippold) was more in comparison to PAA (aqueous latex extract of *Plumeria alba* L.). Whereas, based on the above Table 9 and Figure 3 (c) it was observed that the % inhibition of the CTM (methanolic latex extract of *Cascabela thevetia* (L.) Lippold) was more in comparison to PAM (methanolic latex extract of *Plumeria alba* L.). Hence, it can be said that with the increase in the concentration there was an increase in the trend of antioxidant activity. Further, the IC_{50} values were calculated for finding the best among the latex extracts. Figure 3 (d) represents the IC_{50} values of all four extracts.

The lower the IC_{50} value the greater the antioxidant potential[24]. Therefore, from Figure 3 (d) the order of the IC_{50} observed was PAA> CTA> PAM> CTM. Consequently, the order of the antioxidant potential was CTM> PAM> CTA> PAA. It can be said that among all the latex extracts

the best antioxidant potential was the *Cascabela thevetia* (L.) Lippold methanolic latex extract and the lowest antioxidant potential were of the *Plumeria alba* L. aqueous latex extract.

4. Discussion

Preliminary phytochemical analysis was performed using the aqueous and methanolic latex extracts of the plant's *Plumeria alba* L. and *Cascabela thevetia* (L.) Lippold. Similar phytochemical analysis of the latex extract of *Cascabela thevetia* (L.) Lippold has been reported by Mahajan and Badgujar (2008)[25]. The presence of alkaloids, cyanogenic glycosides, and phenolics and the absence of flavonoids, terpenoids, tannins, and saponins has been reported in the crude latex extracts of the plant respectively. The results of Mahajan and Badgujar 2008 have shown similarities as well as contrasts in accordance with the present study[25]. In the present study, alkaloids have been found completely absent in the aqueous and methanolic latex extracts. The absence of flavonoids, terpenoids, and tannins, has been reported by Mahajan and Badgujar (2008) whereas, in the present study all of these phytochemicals have been found present[25]. In the current study glycosides and phenolics have shown their occurrence in both extracts which is in accordance with the study report of Mahajan and Badgujar (2008)[25]. The presence of saponins has not been reported by Mahajan and Badgujar (2008)[25] whereas, in the present study saponins were found present in the CTA extract and absent in the CTM extract.

FTIR analysis assisted in the detection of the functional group of compounds present in the aqueous and methanolic latex extracts of *Cascabela thevetia* (L.) Lippold and *Plumeria alba* L. In the current study, the classes of compounds that were detected in the PAA extract were alkynes, amines, alkyl halides, aromatic compounds, and carboxylic acids respectively. While in the PAM extract alkanes, alkenes, alkynes, alcohols, amines, aliphatic primary amine, aromatic compounds, carboxylic acids, esters, and ethers were the classes of compounds found respectively.

In the CTA extract, the classes of compounds that were found are as follows- alkynes, amines, alkyl halides, aromatic compounds, and carboxylic acids. While in the CTM extract alkanes, alkynes, alcohols, amines, aliphatic primary amines, aromatic compounds, alkyl halides, carboxylic acids, imine/oxime, esters, and ethers were the classes of compounds found respectively. Reports on the FTIR analysis of silver and gold nanoparticles synthesized from the aqueous latex extracts of *Cascabela thevetia* (L.) Lippold (*Thevetia peruviana*) has been mentioned by Rupiasih *et al*., 2013, Rupiasih *et al.,* 2015. But the reports do not contain the FTIR spectral analysis of the aqueous and methanolic latex extracts separately[26,27].

In latex extracts, a significant amount of phenolics and flavonoid compounds have been found. The highest amount of both these compounds were present in the CTM extract and the lowest in the PAA extract. The phenolic and flavonoid content further reveal these compounds are responsible for the antioxidant activity.

The IC_{50} value of the CTM extract was the lowest which shows that among all the four extracts the antioxidant potential of the CTM extract was the best. The reason for this result is the presence of phenolic and flavonoid content which are present in higher amounts in comparison to the other three extracts. Phenolic compounds are known to exhibit free radical inhibition, decomposition of peroxide, and oxygen scavengers in various biological systems and thus help in preventing the burden of oxidative diseases[28]. Similarly, flavonoids are also known for their antioxidant activity. The number and position of the free-OH groups are responsible for the antioxidant activity of the flavonoids[29].

5. Conclusion

In this study, the assessment of the preliminary phytochemicals suggests the presence of various phytochemical compounds including phenolic compounds and flavonoids. The FTIR analysis revealed the presence of various functional groups and has further shown the differences in functional groups between the latex extracts. These differences can also be the possible reasons for the antioxidant activity. This also proves FTIR spectroscopy can be used as an effective tool for determining the antioxidant properties of the plant latex extracts.

It was seen that the total phenolic content and total flavonoid content were highest in the CTM extract and lowest in the PAA extract. Based on the obtained data we can associate the presence of antioxidant potential of the latex extracts. Further, in the DPPH assay also the best result was obtained from the CTM extract. Finally, our experiment established the presence of antioxidant potential of the aqueous and methanolic latex extracts of *Cascabela thevetia* (L.) Lippold and *Plumeria alba* (L.). The separation of specific phenols and flavonoids are highly encouraged for confirming the antioxidant

properties of the aqueous and methanolic latex extracts of the two plants. As plants are known for their rich cardiac glycosides, the connectivity of antioxidant potential and cardiac glycosides can be possibly found in future research.

6. Acknowledgements

The authors would like to acknowledge the education department of Gujarat State, for the SHODH (ScHeme of Developing High-Quality Research) scholarship, and the financial support.

7. References

1. Endress ME, Liede-Schumann S, Meve U. An updated classification for Apocynaceae. Phytotaxa. 2014; 159(3):175-194. https://doi.org/10.11646/phytotaxa.159.3.2
2. Bhadane BS, Patil MP, Maheshwari VL, Patil RH. Ethnopharmacology, phytochemistry, and biotechnological advances of family apocynaceae: A review. Phytother Res. 2018; 32(7):1181-1210. https://doi.org/10.1002/ptr.6066 PMid:29575195
3. Alvarado-Cárdenas LO, Ochoterena H. A Phylogenetic analysis of the *Cascabela-thevetia* species complex (plumerieae, apocynaceae) based on morphology. Ann Mo Bot Gard. 2007; 94(2):298-323. https://doi.org/10.3417/0026-6493(2007)94[298:APAOTC]2.0.CO;2
4. Morales JF. La Familia Apocynaceae (Apocynoideae, Rauvofioideae) En Guatemala. Darwiniana. 2009; 47(1):140-184. https://doi.org/10.14522/darwiniana.2014.471.50.
5. Naz S, Sulthana S. An Updated review on the Genus Cascabela plant family: Apocynaceae. Int J Pharm Chem Sci. 2013; 2(3):1474-1477.
6. Alvarado-Cárdenas LO. A new species of *Cascabela* (Apocynaceae; Rauvolfioideae, Plumerieae) from Michoacán, Mexico. Phytotaxa. 2014; 177(3):163-170. https://doi.org/10.11646/phytotaxa.177.3.4. https://doi.org/10.11646/phytotaxa.177.3.4
7. González-Rocha E, Cerros-Tlatilpa R. La familia apocynaceae (Apocynoideae Y Rauvolfioideae) en el estado de morelos, México. Acta Bot Mex. 2015; 110(1):21-70. https://doi.org/10.21829/abm110.2015.191
8. Williams JK, Stutzman JK. Chromosome number of *Thevetia ahouai* (Apocynaceae: Rauvolfoidae: Plumerieae) with discussion on the generic boundaries of *Thevetia*. J Bot Res Inst Tex. 2008; 2(1):489-493. https://doi.org/10.21829/abm110.2015.191
9. Zarucchi JL. Thevetia. In: Davidse G, Sousa SM, Knapp S, Chiang F, editors. *Flora mesoamericana*. 1st ed. London. The Natural History Museum; 2009.
10. Shivaraja A, Appaji S, Sudha R.V.R. Suicide tree poisoning. Int J Contemp Pediatr. 2018; 5(4):1690-1692. https://doi.org/10.18203/2349-3291.ijcp20182591
11. Sundaragiri S, Tandur S. Electrocardiographic profile of cardiotoxic plants and animals. Int J Med Res Health Sci. 2016; 5(11):719-725.
12. Pasindla B, Rani SJ, Reddy SR, Supriya N, Bakshi V, Boggula N. Phytopharmacological importance of *Thevetia peruviana*: A literature-based review. Int J Pharmacol Pharm Res. 2019; 1(1):33-37.
13. Rajhans S, Pandya H, Mankad A. A comprehensive review on globally present cardiotoxic plants. Toxicol Int. 2021; 28(4):385-401. https://www.doi.org/10.18311/ti/2021/v28i4/28028.
14. Choudhary M, Kumar V, Singh, S. Phytochemical and pharmacological activity of genus plumeria: An updated review. Int J Biomed Adv Res. 2014; 5(6):266-271. https://doi.org/10.7439/ijbar.v5i6.761.
15. Imran M, Asif M. Morphological, ethnobotanical, pharmacognostical and pharmacological studies on the medicinal plant *Plumeria alba* Linn. (Apocynaceae). Arab J Med Aroma Plants. 2020; 6(1):54-84.
16. Aliyu RM, Abubakar MB, Kasarawa AB, Dabai YU, Lawal N, Bello MB, Fardami, AY. Efficacy and phytochemical analysis of latex of *Calotropis procera* against selected dermatophytes. J Intercult Ethnopharmacol. 2015;4(4):314-317. https://doi.org/10.5455/jice.20151012012909 PMid:26649237 PMCid:PMC4665027
17. Banu KS, & Cathrine L. General techniques involved in phytochemical analysis. Int J Adv Res Chem Sci. 2015; 2(4), 25-32.
18. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A Review. Int Pharm Sci. 2011; 1(1):98-106.
19. Velavan S. Phytochemical techniques- a review. World J Sci Res. 2015; 1(2):80-91.
20. Song K. Interphase characterization in rubber nanocomposites. In: Thomas S, Maria HJ, editors. Progress in Rubber Nanocomposites. 1st ed. UK: Woodhead Publishing; 2017. https://doi.org/10.1016/B978-0-08-100409-8.00004-8
21. Saini V, Mishra R. Quantitative estimation of total phenolic and flavonoid compounds of medicinal plants *Jatropha gossypiifolia* and *Tagetes erecta*. World J Pharm Res. 2018; 7(1):1137-1147.
22. Dubey R, Rajhans S, Mankad A. preliminary phytochemical screening, quantitative estimation of total phenolic and flavonoid content of *Jatropha gossypiifolia* (L.). Res J Pharm Phytochem. 2020; 12(2):1-4. https://doi.org/10.5958/0975-4385.2020.00015.1
23. Mata R, Nakkala JR, Sadras, SR. Catalytic and biological activities of green silver nanoparticles synthesized from

Plumeria alba (frangipani) flower extract. Mat Sci Eng C. 2015; 51(1):216-225. https://doi.org/10.1016/j.msec.2015.02.053. https://doi.org/10.1016/j.msec.2015.02.053 PMid:25842128

24. Rivero-Cruz JF, Granados-Pineda J, Pedraza-Chaverri J, Perez-Rojas JM, Kumar-Passari A, Diaz-Ruiz G, & Rivero-Cruz BE. Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican Brown Propolis. Antioxidants. 2020; 9(70):1-11. https://doi.org/10.3390/antiox9010070 PMid: 31940981 PMCid:PMC7022611
25. Mahajan RT, Badgujar SB. Phytochemical investigations of some laticiferous plants belonging to Khandesh region of Maharashtra. Ethnobot Leaf. 2008;12(1):1145-1152. https://opensiuc.lib.siu.edu/ebl/vol2008/iss1/151.
26. Rupiasih NN, Aher A, Gosavi S, Vidyasagar, PB. Green synthesis of silver nanoparticles using latex extract of *Thevetia peruviana*: A novel approach towards poisonous plant utilization. J Phy: Conf Ser. 2013; 423(1):1-8. https://doi.org/10.1088/1742-6596/423/1/012032
27. Rupiasih NN, Aher A, Gosavi S, Vidyasagar PB. *Thevetia peruviana*: A wild natural resource for the green synthesis of gold nanoparticles. Adv Mat Res. 2015; 1112(1):71-75. https://doi.org/10.4028/www.scientific.net/AMR.1112.71
28. Babbar N, Oberoi HS, Sandhu SK. Therapeutic and nutraceutical potential of bioactive compounds extracted from fruit residues. Cr Rev Food Sci Nutri. 2014; 55(3):319-337. https://doi.org/10.1080/10408398.2011.653734 PMid: 24915390
29. Panche AN, Diwan AD, Chandra SR. Flavonoids: An Overview. J Nutri Sci. 2016;5(e47):1-15.https://doi.org/10.1017/jns.2016.41 PMid:28620474 PMCid:PMC5465813