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EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND PROTEASE CHARACTERIZATION FROM LATEX OF *PLUMERIA ALBA, EUPHORBIA GRANTII* AND *CALOTROPIS GIGANTEA*

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Keywords:

Latex, Antioxidant activity, Antimicrobial activity, Protease, *Calotropis gigantea*

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ABSTRACT: Latex-bearing plants represent a rich source of bioactive compounds with significant medicinal value with wound healing property, antimicrobial activity, antiinflammatory and anticancer activity. The present investigation was aimed at evaluating the antioxidant activity, protease characterization and antimicrobial activity of latex extracts of *Plumeria alba*, *Euphorbia grantii* and *Calotropis gigantea*. Phytochemical analysis revealed that Calotropis gigantea latex contained the highest phenolic content, while *Plumeria alba* showed maximum protein and carbohydrate content. Antioxidant assays such as DPPH radical scavenging, reducing power assay and lipid peroxidation inhibition demonstrated strong antioxidant activity, particularly in *Calotropis gigantea* latex. Antimicrobial screening against *Staphylococcus aureus* using agar well diffusion method showed the highest zone of inhibition for *Calotropis gigantea*. Protease activity was maximum for *Euphorbia grantii* with optimal pH 8, with temperature optima of 40°C for *Plumeria alba* and *Euphorbia grantii* and 60°C for *Calotropis gigantea*. These findings support the traditional medicinal use of plant latex and suggest its potential application in pharmaceutical and industrial fields.

INTRODUCTION: Plant latex is a complex biological fluid secreted by specialized cells called laticifers and is widely distributed among angiosperms [1]. Latex contains a variety of secondary metabolites including alkaloids, phenolics, flavonoids, terpenoids, sugars and proteins, which contribute to plant defense mechanisms.

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Reactive oxygen species generated during metabolic processes induce oxidative stress, leading to cellular damage and chronic diseases. Natural antioxidants derived from plants play a crucial role in neutralizing free radicals and inhibit the free radical mediated diseases. The plant latex has many enzymes that have the defensive role to protect from herbivores, insects, fungi and bacteria [2].

Proteases helps in wound healing, blood clotting, antimicrobial activity, antiinflammatory, anticancer activity, vermicidal, anthelmintic activity and blood clot dissolving activity [3]. Plant latex proteases mimic thrombin and trigger blood clot formation. Many latex proteases also show clot dissolving

potential like plasmin [4]. It is used as digestive aid, anti-inflammatory and analgesic, in industry for food processing and pharmaceuticals [5].

This study investigates phytochemical analysis, antioxidant activity, antimicrobial activity and protease characterization of *Plumeria alba*, *Euphorbia grantii* and *Calotropis gigantea* latex. These small bioactive compounds and peptides in latex can be used in therapeutics for bacterial infections against rising antibiotic resistance.

MATERIALS AND METHODS: Diphenyl picryl hydrazyl radical (DPPH), thiobarbituric acid, gallic acid, quercitin were purchased from SRL company. All other chemicals and reagents purchased were of analytical grade. Latex samples were collected freshly from *Plumeria alba*, *Euphorbia grantii* and *Calotropis gigantea* plants.

Preparation of Latex Sample: The latex samples were collected from three different plants and stored at -4°C before starting the experiments and later it is thawed and used. The samples were diluted using phosphate buffer (0.2 M, pH 7.0) followed by centrifugation and kept overnight at 4°C. The sample was centrifuged at 5000rpm for 10 minutes. After centrifugation the supernatant was collected and that constitutes crude sample for protease activity, analysis of proteins, sugars, phytochemicals, antioxidant activity and antimicrobial activity [6, 7].

Estimation of Phenolics: The concentration of total phenolics in all the extracts was determined by the Folin-Ciocalteu assay. It involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725 nm increases linearly with the concentration of phenolics in the reaction medium [8]. In this study Gallic acid was used as spectrophotometric standard. The phenolic contents of the latex samples were determined from calibration curve and were expressed in mg of Gallic acid equivalents/ ml sample.

Estimation of Total Flavonoid Content: Aluminium chloride colorimetric method [9] was used for flavonoids determination. The absorbance of the reaction mixture was measured at 510 nm versus a blank. Quercitin was used as standard for the calibration curve.

Total flavonoid concentration of the latex samples were expressed as mg of quercitin equivalents per ml of sample.

Estimation of Total Protein Content: The total protein content was estimated using biuret method [10]. The peptide bond in the polypeptide chain reacts with copper sulphate in an alkaline medium to give a purple colour which can be measured at 540nm. The calibration curve was plotted using standard gelatin solution. The total protein content was expressed in terms of mg/ml of sample.

Estimation of Total Sugar Content: Total sugars were estimated according to the procedure of phenol-sulphuric acid method [11]. Total sugars were expressed in terms mg of glucose/ ml of sample. Glucose (0.2-1 ml) was used as reference standard.

Screening of Antioxidant Activity:

DPPH Radical Scavenging Assay: Determination of antioxidant activity by the DPPH method [12] was done for all the latex samples. Diphenyl-1-picryl hydrazyl (DPPH) was used as a stable radical for assessing antioxidant activity. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorption at 517 nm.

Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Percentage of radical scavenging activity was calculated for all the three samples.

Reducing Power Assay: The substances in the samples reduces potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), which then react with ferrichloride ($FeCl_3$) to form ferric-ferrous (Fe^{3+}-Fe^{2+}) complex showing absorbance at 710nm. The reducing power of all the latex samples was evaluated according to the method of Oyaizu [13].

Lipid Peroxidation Assay (TBA Assay): Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm as per Halliwell and Gutteridge [14]. The egg yolk was used for the study of *in-vitro* lipid peroxidation. The percentage of anti lipid peroxidative activity (%ALP) is calculated for all extracts.

Percentage of antilipid peroxidation = (Control-Test) / Control × 100

Protease Assay:

Protein Estimation: Protein concentration of soluble enzyme preparation was quantified by Lowry's method using bovine serum albumin (BSA) as a standard [15].

Protese Activity (Caseinolytic Activity): Caseinolytic activity were carried out for this determination, the reaction mixture contained 0.4ml of casein (2%) in 0.2M Tris-HCl buffer of pH 8.5 was incubated separately with different plant latex samples for 2 hours at 37°C. The reaction was stopped by adding 1.5ml of 0.44M TCA and the mixture was allowed to stand for 30 min. The samples were centrifuged at 1500xg for 15 minutes. To the 1ml of supernatant add 2.5 ml 0.4 M Sodium carbonate and 0.5 ml of Folin & Ciocalteus Phenol reagent (1:2, v/v). Optical density of blue colour that develops was measured at 660 nm. One unit of caseinolytic activity was defined as the amount of enzyme required to increase an absorbance of 0.01 at 660nm/h at 37 degree Celsius. Activity was expressed as units/h at 37°C [16].

Protease Characterization:

Effect of Temperature: For the determination of optimum temperature of protease in three different latex samples, protease was assayed at temperatures ranging between 0 to 100°C in a temperature controlled water bath using the standard method of assay.

Effect of pH: Optimum pH of protease activity in three different latex samples was determined covering the range 3 to 9 using 0.2M buffers of different pH. The buffers were; pH 3 to 6 (citrate), pH 7 (phosphate), pH 8 to 9 (Tris-Hcl). The activity of different pH was determined using standard assay method.

Effect of Substrate Concentration: Enzyme kinetic studies were carried out to determine the enzyme substrate affinity. The Michaelis-Menten method is the simplest and effective way for enzyme kinetic study.

Protease activity assayed in reaction buffer at 37°C with different concentrations of casein as a substrate. The values of Vmax (maximum velocity) and Km (Michaelis constant) were calculated from Lineweaver-Burk plot.

Antimicrobial Activity (Agar well Diffusion Method):

Test Microorganisms: The aqueous extract of the latex of *Calotropis gigantea, Plumeria alba* and *Euphorbia grantii* was tested against pathogenic bacteria *S. aureus*. All the cultures were isolated from the laboratory samples. All the test organisms were inoculated into nutrient broth and incubated at 37°C for hours.

Positive and Negative Control: Penicillin G disc (10 µg/disc) was used as positive control (PC) for *S. aureus* and phosphate buffer was used as negative control (NC).

Antimicrobial Assay: Antimicrobial activity of the crude latex extract was determined by agar well diffusion method [17]. The bacterial suspensions were seeded on nutrient agar plates. In each of these plates two wells were cut out using a sterilize cork borer. Using a micropipette, 30,50, 70 µl of crude extract and negative control was added in to different wells. A positive control antibiotic disc was placed in the plate. Bacterial plates were incubated for 72 hours at room temperature. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition.

RESULTS AND DISCUSSION: The latex samples of *Calotropis gigantea, Plumeria alba*, and *Euphorbia grantii* were evaluated for their phytochemical constituents, antioxidant potential, protease activity and characterization, and antimicrobial activity. The results obtained are summarized in **Table 1** and **2**.

Among the three samples, *Calotropis gigantea* latex exhibited the highest total phenolic content (2.6 mg/ml), while *Plumeria alba* latex showed the highest flavonoid content (0.29 mg/ml), expressed as quercetin equivalents as depicted in **Fig. 1** & **2**. In addition, *Plumeria alba* latex demonstrated maximum protein (50 mg/ml) and total sugar (6.2 mg/ml) contents, indicating a rich biochemical composition as shown in **Fig. 3** & **4**.

The antioxidant potential of the latex samples varied significantly. The DPPH radical scavenging activity was highest in *Calotropis gigantea* latex (34%), followed by *Euphorbia grantii* (22%) and *Plumeria alba* (5%) is shown in **Fig. 5.** A similar trend was observed in the reducing power assay,

where *Calotropis gigantea* showed the highest absorbance (1.38 at 700 nm), compared to *Plumeria alba* (0.46) and *Euphorbia grantii* (0.10) as depicted in **Fig. 6.**

These results suggest a strong electron-donating ability of *Calotropis gigantea* latex, which may be attributed to its higher phenolic content. In contrast, the antilipid peroxidation assay revealed that *Plumeria alba* latex exhibited the highest inhibition (73%), followed by *Euphorbia grantii* (63%) and *Calotropis gigantea* (57%) as shown in **Fig. 7**.

This variation indicates that different antioxidant mechanisms may be influenced by distinct phytochemical profiles present in the latex samples. Protease activity analysis showed that *Euphorbia grantii* latex possessed the maximum proteolytic activity (13.6 units/hr) depicted in **Fig. 8.**

The optimum temperature for protease activity was observed at 40 °C for *Plumeria alba* and *Euphorbia grantii*, while *Calotropis gigantea* exhibited optimal activity at 60 °C **Fig. 9.** The stability and activity of these proteases under alkaline conditions suggest their potential suitability for industrial applications, particularly in detergent and pharmaceutical industries **Fig. 10.**

The enzyme samples of three different latex shows maximum activity at a time 120 min **Fig. 11.** The antimicrobial activity of all three latex samples was assessed against *Staphylococcus aureus*. The aqueous latex extract of *Calotropis gigantea* demonstrated the highest zone of inhibition (19 mm), whereas *Plumeria alba* and *Euphorbia grantii* showed comparatively lower inhibition zones of 6 mm and 5 mm, respectively **Table 2**.

Infectious diseases caused by microorganisms remain a major cause of morbidity and mortality worldwide. Although antibiotics have been developed with considerable efficacy, their misuse and overuse, along with microbial mutations, have led to the emergence of multidrug-resistant strains. Consequently, many conventional antibiotics have gradually lost their effectiveness.

In this context, the significant antibacterial activity observed in *Calotropis gigantea* latex may be correlated with its higher phenolic and flavonoid contents, which are known to disrupt microbial cell membranes and inhibit essential enzymatic processes.

Overall, *Calotropis gigantea* latex exhibited superior biological activities among the three samples studied, supporting its extensive use in traditional medicine and highlighting its potential as a natural source of bioactive compounds for therapeutic and industrial applications.

TABLE 1: PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF THREE DIFFERENT VARIETIES OF PLANTS LATEX

Source	*Plumeria alba*	*Euphorbia grantii*	*Calotropis gigantea*
Aqueous solution(1ml of sample)			
Phenolics (mg/ml of sample)	2.4	0.9	2.6
Flavonoids (mg/ml of sample)	0.29	0.19	0.25
Proteins (mg/ml of sample)	50	10	26
Sugars (mg/ml of sample)	6.2	3.6	3
Antioxidant activity (DPPH assay) % of radical scavenging activity	5	22	34
Reducing power assay (absorbance at 700 nm)	0.46	0.10	1.38
TBA assay (% of antilipid peroxidation)	73	63	57

TABLE 2: ANTIMICROBIAL ACTIVITY OF LATEX EXTRACT OF *PLUMERIA ALBA, EUPHORBIA GRANTII, CALOTROPIS GIGANTEA* ON TESTED ORGANISMS (*STAPHYLOCOCCUS AUREUS*)

Sample	Zone of inhibition in (mm)		
	Latex	**PC**	**NC**
Plumeria alba	6	25	-
Euphorbia grantii	5	25	-
Calotropis gigantea	19	25	-

PC- positive control; NC- negative control.

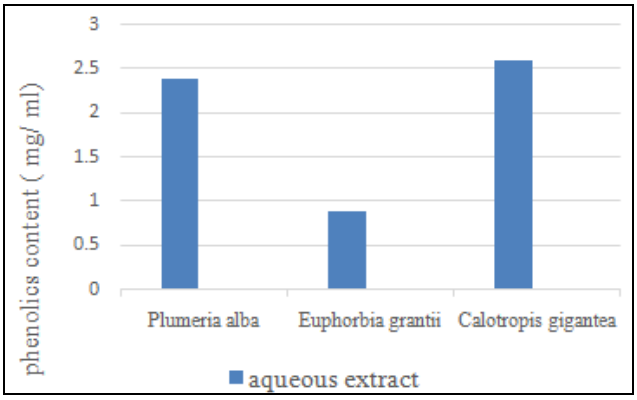

FIG. 1: TOTAL PHENOLICS IN PLANT LATEX SAMPLES

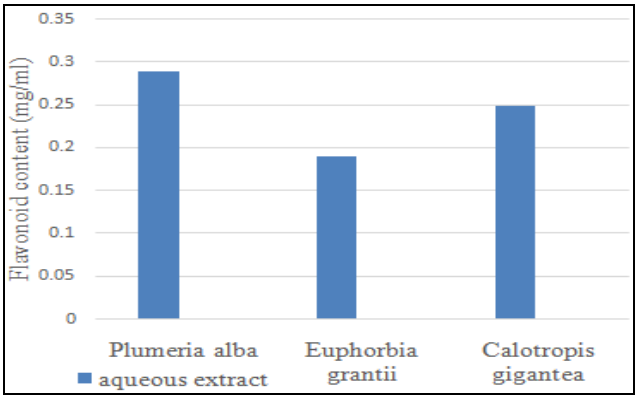

FIG. 2: TOTAL FLAVONOID CONTENT IN PLANT LATEX SAMPLES

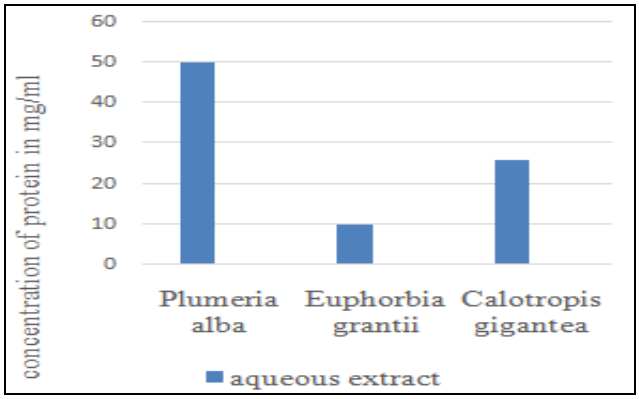

FIG. 3: TOTAL PROTEIN CONTENTS IN PLAN LATEX SAMPLES

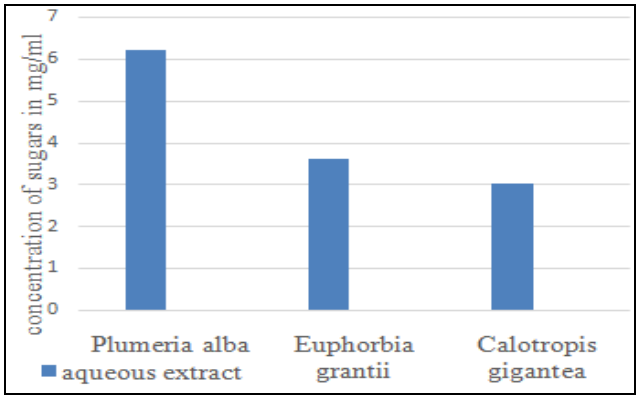

FIG. 4: TOTAL SUGAR CONTENTS IN PLANT LATEX SAMPLES

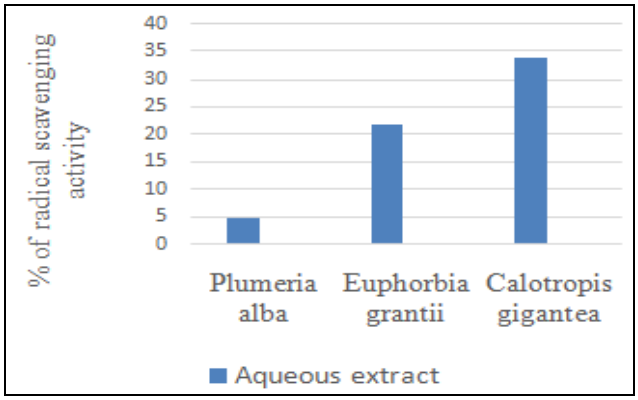

FIG. 5: PERCENTAGE OF RADICAL SCAVENGING ACTIVITY IN PLANT LATEX SAMPLES

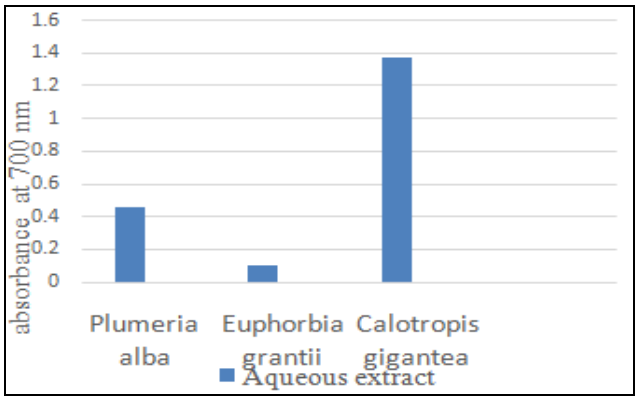

FIG. 6: REDUCING POWER ACTIVITY IN PLANT LATEX SAMPLES

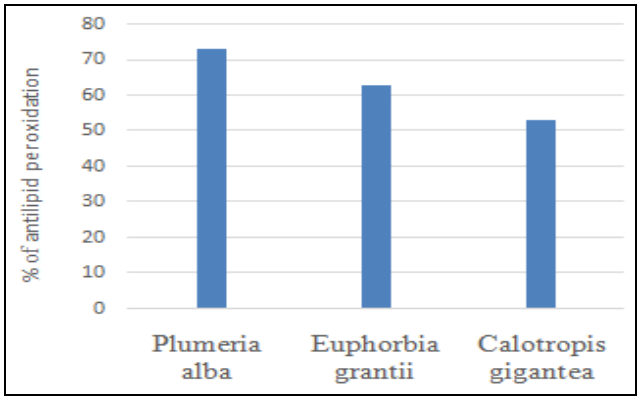

FIG. 7: PERCENTAGE OF ANTILIPID PEROXIDATION ACTIVITY IN PLANT LATEX SAMPLES

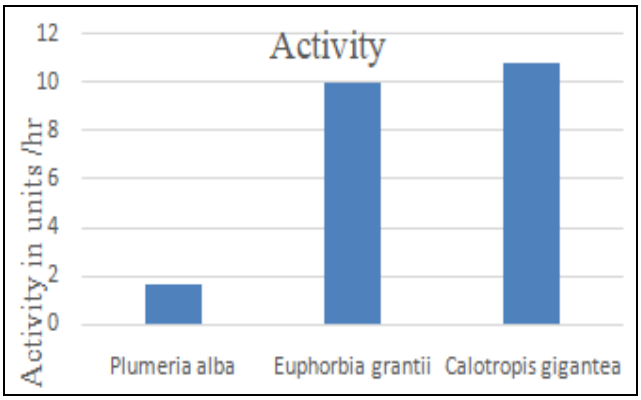

FIG. 8: PROTEASE ACTIVITY IN PLANT LATEX SAMPLES

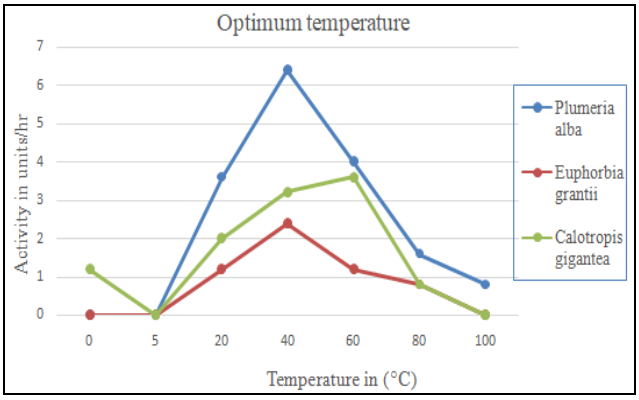

FIG. 9: OPTIMUM TEMPERATURE ACTIVITY IN PLANT LATEX SAMPLES

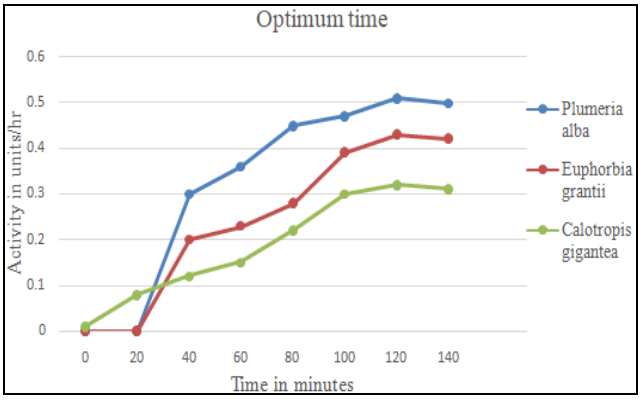

FIG. 10: OPTIMUM PH ACTIVITY IN PLANT LATEX SAMPLES

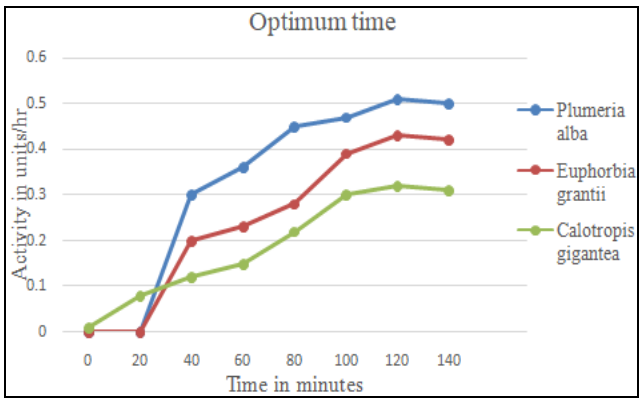

FIG. 11: OPTIMUM TIME ACTIVITY IN PLANT LATEX SAMPLES

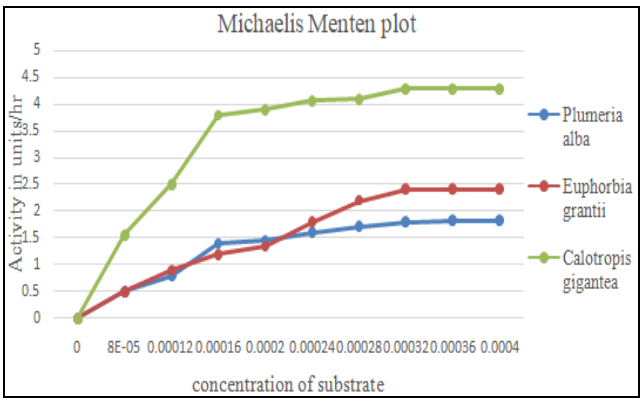

FIG. 12: EFFECT OF SUBSTRATE CONCENTRATION IN PLANT LATEX SAMPLES (MM PLOT)

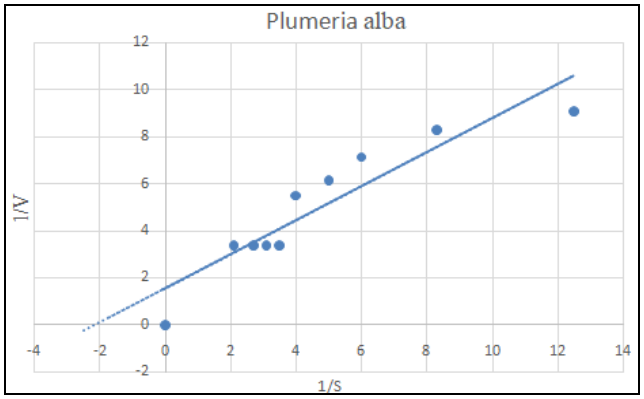

FIG. 13: EFFECT OF SUBSTRATE CONCENTRATION IN *PLUMERIA ALBA* PLANT LATEX SAMPLE (LB PLOT)

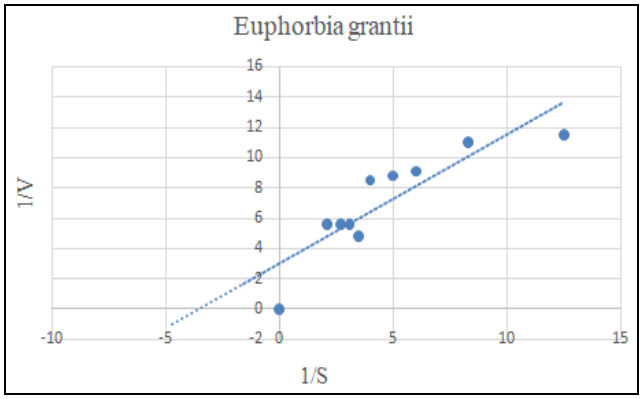

FIG. 14: EFFECT OF SUBSTRATE CONCENTRATION IN *EUPHORBIA GRANTII* PLANT LATEX SAMPLE (LB PLOT)

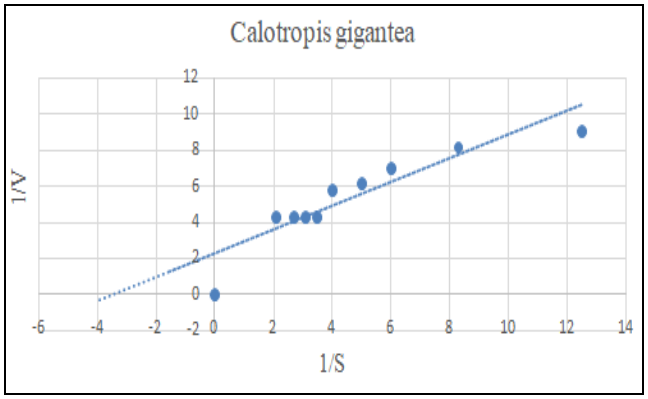

FIG. 15: EFFECT OF SUBSTRATE CONCENTRATION IN *CALOTROPIS GIGANTEA* PLANT LATEX SAMPLE (LB PLOT)

CONCLUSION: This study demonstrates that latex extracts of *Plumeria alba*, *Euphorbia grantii* and *Calotropis gigantea* posses substantial antioxidant, antimicrobial and protease activities. *Calotropis gigantea* latex shows the highest potential and further investigation for isolation of active compounds for medicinal, pharmaceutical and industrial applications.

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