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ANTIOXIDANT ANALYSIS AND ENZYME INHIBITION ASSAY OF PLANTS OF APOCYNACEAE FAMILY FOR ULCERS

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Keywords:

Antioxidant activity, Apocynaceae, β- carotene bleaching assay, Flavonoids, Urease inhibition

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ABSTRACT: Different plants of the Apocynaceae family *viz. Alstonia scholaris, Calotropis procera, Rauvolfia serpentine, Hemidesmus indicus* and *Calotropis gigantean* are traditionally being reported in ulcers treatments. The present study was aimed to validate the authenticity of correlationship of various phytoconstituents concentrations, antioxidant activity and urease inhibitory potential of the same. Various methods of antioxidant potential possess variable modes of deactivation of free radicals. Plants possess myriad of chemical entities, an effort has been made to assign antioxidant and urease inhibitory putativity to various classes of compounds. The Flavonoid content seemed significant for antioxidant as well urease inhibitory potential. *Calotropis procera* and *Calotropis gigantean* possessed 104.11mgRU/g and 97.34mgRU/g of flavonoids. The two exhibited 39% and 40% urease inhibitory potential respectively. The ferric reducing power and the β- carotene bleaching tendency were highest in both species of Calotropis genus. Pearson's correlation coefficient between total flavonoids-urease inhibitory percentage, β-carotene bleaching - flavonoids and FRAP - flavonoids content was significant. So the plants of Calotropis genus have good potential for the management of ulcers, gastric disease and related conditions of oxidative stress.

INTRODUCTION: Medicinal plants are of great importance to the health of mankind in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. The plants of the genus Apocynaceae family are widely distributed and have long been used in folk medicine for the treatment of various ailments such as meningitis in children and rheumatic, heart diseases, hernia, infantile malnutrition, dyspepsia and testitis [1, 2].

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Over 100 alkaloids of family have been identified and evaluated for various biological activities [3]. Some are sources of important drugs, such as cardiac glycosides, affect heart function [2]. This study is an attempt to analyze chemistry, antioxidant activity and urease inhibitory potential of the five Indian native plants of the family Apocynaceae *viz. Alstonia scholaris, Calotropis procera, Rauvolfia serpentia, Hemidesmus indicus* and *Calotropis gigantean.*

Oxidation happens from everyday body functions plus toxic substances, and sunlight. This oxidative stress leads to the induction of many chronic and degenerative diseases like Alzheimer's disease, Parkinson's disease, cancers, diabetes mellitus, ageing, immune suppression, inflammatory diseases, neurodegenerative diseases, cardiovascular diseases, rheumatoid arthritis, neurodegenerative diseases, asthma, cataract, liver damage, ulcerative colitis [4].

Antioxidant is a chemical compound or substance that inhibits oxidation. They work to keep our cells healthy by protecting them from damage by free radicals. Antioxidants help repair damaged cells, which can prevent diseases, including cancer. Urease serves as a malignant factor in microbes that are the main cause of peptic ulcers, kidney stones formation, pyelonephritis and other malfunctioned states. The search for the photochemical inhibitors of urease raises the possibility to explore chemical entities as modulators and inhibitors of urease activities. Commercially available urease inhibitors are hydroxamic acid, imidazole derivatives, and bismuth complexes [5]. Researchers have revealed many other compounds such as phosphoramides, heterocyclic compounds, quinones, heavy metal ions and thiols act as urease inhibitors. 1, 2-Benzisoselenazol-3(2H) - one derivatives also represent the new class of new bacterial urease inhibitor [6]. But they exhibit some unfavourable features like low hydrolytic stability and toxicity, which alter their clinical use.

For Agricultural interest NBPT (N-(butyl) thiophosphoric triamide) is commercially available effective soil urease inhibitor but it is directly related to soil properties. Till now, phosphorodiamide and phosphorotriamide derivatives are used as most efficient for crop production purposes. Plant phenolic compounds such as quercitrin, avicularin, flavonoid glucosides and shoreaphenol are also reported to inhibit Jack bean urease [7]. So search for new and effective inhibitors of urease is needed.

Natural flora possesses structural and functional diversity and hence an ocean of therapeutic agents. Various phytochemicals, such as coumarins, flavonoids, polyphenolic compounds, xanthones, *etc*., have widely been reported for many biological activities such as anti-inflammatory, radical scavenging ability, hepatoprotective, anti-cancer, anti-bacterial, and anti-viral properties and hence seem likely to play an important role in the development of new therapeutic agents against urease related diseases [8]. Commercially available natural flavonoids have exhibited an excellent urease inhibitory activity [9].

The present study was designed to validate correlation of oxidative potential, quantitative availability of flavanoids with the urease inhibitory potential, among Indian native plants of family Apocynaceae (**Table 1**). In this regard, ten extracts were subjected to photochemical analysis, *in vitro* antioxidant properties and urease inhibition assay. The active site of Jack Bean (*Canavalia ensiformis*) urease (EC 3.5.1.5), resembles that of bacterial ureases. It is therefore assumed that inhibitors of Jack Bean urease will also inhibit the ureases of bacterial origin. The combinational effect of antioxidant potential and urease inhibition in plants contribute many folds to the benefit of health.

TABLE 1: LIST OF THE PLANTS TO BE STUDIED

S. no.	Botanical Name	Local name	Traditional uses	Reported biological activities
1	*Alstonia scholaris*	Saptaparna	Urinary diseases, poisoning, fever, malignant ulcers, leprosy, virulent skin diseases	Anti-inflammatory, analgesic [10], Antiulcer [11], Antibacterial [12], Anticancer [13]
2	*Calotropis procera*	Safed Arka	Bronchodilator, arthritis and skin diseases	Anti-inflammatory [14], Antiulcer [14, 15], Anti-microbial [16], Anticancer [17], Antioxidant [18]
3	*Rauvolfia serpentina*	Sarpagandha	Antihypertensive, tranquilizer.	Anti-diarrhoeal [19], Anti-hypertensive [20]
4	*Hemidesmus indicus*	Anantamul	Blood purifier, ulcers, fever, loss of appetite, gastritis, menorrhagia, diarrhoea and diabetes	Anti-arthritic [21], Cure ulcers [22]
5	*Calotropis gigantean*	Arka	Skin diseases, Bloating, used in indigestion and chronic diarrhoea used in cough, cold, backache, stiffness, diabetes	Anti- microbial, Anti-oxidant [23], Anti-inflammatory, Immune restorative [24], Antibacterial [25], Anti-ulcer [26]

MATERIALS AND METHODS:

Chemicals: Urease type 1X (specific activity: 50,000-1,00,000units/g) from *Canavalia ensiformis* L. DC. (Fabaceae) commonly known as Jack-Bean was purchased from Sigma Aldrich (St. Louis, MO). 2, 2´ diphenyl- 1- picrylhydrazyl, Folin-

ciocalteu's reagent, ascorbic acid, trichloroacetic acid, and glacial acetic acid were purchased from Hi Media Pvt. Ltd. All other chemicals and reagents used in this study were of analytical grade.

Plant Material and Preparation of Extract: Leaves of the selected flora were collected from surroundings of Kurukshetra University, Kurukshetra, Haryana, India (29° 57' 31.353" N, 76° 48'5 2.128" E). The identifications and authentication of the specimens was done from the Department of Botany, Kurukshetra University, Kurukshetra. The collected leaves were washed under tap water followed by distilled water, shade dried for 7 days and then milled to a coarse powder by a mechanical grinder and stored till the further use.

The powders of dried leaves (10g) were separately soaked in distilled water and methanol (100ml) in a reagent bottle covered with a lid at 37 °C for 24h. The powder were packed into soxhlet column for 48 h. Resulting extracts in different solvents were evaporated and concentrated to dryness using the rotatory evaporator at 50 °C. The yield of extraction of dried plant material was calculated. The extracts were stored at -4 °C.

Preliminary Phytochemical Screening: Both extracts of the selected plants were evaluated for the screening of the various phytochemicals using standard phytochemical procedures [27].

Estimation of Total Phenolic Content: The content of total phenols was determined according to the Folin - Ciocalteu's method [28]. Aliquots of 0.5mL of extracts were mixed with 2.5mL of 10-fold-diluted Folin - Ciocalteu reagent and 2mL of 7.5% sodium carbonate (Na_2CO_3). The mixture was allowed to stand for 90 min at room temperature before the absorbance taken at 760nm spectrophotometrically. The final results were calibrated to deduct the contribution from ascorbic acid and expressed as Gallic acid equivalent (mg of GA/g of extract).

Estimation of Total Flavonoid Content: The content of flavonoids in the examined plant extracts was determined using spectrophotometric method by Quettier [29]. A volume of 2.5mL of extract was transferred to a test tube, mixed with 0.15mL of 5% Sodium nitrite for 5min. Then, 0.15mL of 10% aluminium nitrate was added. After 6mins the reaction stopped by adding 1mL of 1M sodium hydroxide. The mixture was further diluted with distilled water up to 5mL. The absorbance of the mixture immediately measured at 510nm. The flavonoid content was calculated and expressed as rutin equivalent (mg of RU/g of extract).

Estimation of Total Tannin Content: Total Tannin content was measured by Folin - Ciocalteu's method [30]. 7.5ml of distilled water was added in 0.1ml of the extracts followed by addition of 0.5ml Folin - Ciocalteu reagent. 1ml of 35% Na_2CO_3 was added to the above mixture then it was diluted upto 10ml with distilled water. Mixture was kept at room temperature for 30 minutes and absorbance was taken at 725nm. The final results were calibrated to deduct the contribution from ascorbic acid and expressed as Gallic acid equivalent.

Estimation of Ascorbic Acid: Ascorbic acid content was determined by using method of Roe and Keuther [31]. Briefly, ascorbic acid was oxidized by activated charcoals to yield dehydroascorbic acid, which further react with 2, 4-dinitro-phenylhydrazine to form osazone, a light-absorbing substance. The absorbance read at 490nm spectrophotometrically. Ascorbic acid was taken as standard and the levels of ascorbic acid in the sample expressed as mg ascorbate / g sample.

***In vitro* Antioxidant Evaluation:**

2, 2-diphenyl1picrylhydrazyl Scavenging Assay: The ability of the plant extract to scavenge DPPH free radicals was assessed by the method described by Mensor [32]. The L-ascorbic acid was used as a positive control. The reaction mixture was prepared containing 300µl of extract of varying concentrations (1-100µg/ml) and 2ml of DPPH (0.1mM in methanol). The reaction mixture was then placed in the cuvette holder of the spectrophotometer and the absorbance measure at 517nm against the blank. The per cent DPPH decolourization of the sample was calculated by the equation: % inhibition = B_0-B_1 / B_0 × 100, Where, B_0 is the absorbance of negative control and B_1 is the absorbance of reaction mixture.

Chelating Effects on Ferrous Ions: The measurement of meal chelating activity of the different extracts was estimated by the method of Dinis [33]. The extracts (0.25ml) were mixed with

1.75ml of methanol and 0.25ml of 250mM $FeCl_2$. This was followed by the addition of 0.25ml of 2mM ferrozine, and kept at room temperature for 10 min. before determining the absorbance of the mixture at 562nm. The chelating effect (%) was calculated from the formula as given for DPPH scavenging assay.

Ferric Reducing Antioxidant Power Assay (FRAP): FRAP assay of the extracts was determined by a method based on the reduction of ferric-tripyridyltriazine complex to a blue coloured ferrous form described by Benzie and Strain [34]. The FRAP reagent containing 2.5mL of 10mM 2,4,6-tripyridy-s-triazine solution in 40mM HCl and 2.5mL of 20mM $Fecl_3$ and 25mL of 0.3M acetate buffer, pH 3.6, and prepared freshly and pre-warmed at 37 °C. Aliquots of 40µL of extracts mixed with 0.2mL of distilled water and 1.8mL of FRAP reagent. The absorbance of reaction mixture at 593nm was measured spectrophotometrically after incubation at 37 °C. In this assay, the final result expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1mM $FeSO_4$.

Coupled Oxidation of β-carotene and Linoleic Acid: β-carotene linoleic acid assay of the listed plants was carried out as described by Miller [35]. BHT was used for comparison. 2mg of β-carotene dissolved in chloroform (10ml) was pipetted into a small, round-bottom flask.

After the chloroform was removed at 40 °C under vacuum, 100µl of linoleic acid, 400mg of Tween 20 and 100ml of oxygenated water was added to the flask followed by vigorous mechanical stirring. 5ml of the above prepared emulsion was transferred to a series of test tubes containing 200µl of extracts of various concentrations. As soon as the emulsion was added to each tube, the zero time absorbance is measured at 470nm using a spectrophotometer (T60 UV visible). After initial vortexing, the test tubes were incubated in a water bath at 50 °C for 120 min. The absorbance of each sample was measured at 470nm. Blank, devoid of β-carotene was prepared for background subtraction.

Antioxidant activity calculated using the following equation:

$$AA = 100\{1-A_o - A_t / A_o^o - A_t^o\}$$

Where, A_o and A_o^o are the absorbance at zero time of test and blank, while A_t and A_t^o are the absorbance at time t of test and blank respectively.

Urease Inhibition Studies: The enzyme activity and inhibition was measured through catalytic effects of urease on urea by measuring change in absorbance in the absence and in the presence of inhibitor at 640nm, using spectrophotometer (T60 UV visible). All phytochemicals were tested for urease inhibition activity at concentration of 1.0 mg/ml. and that exerted significant inhibition, and tested in a concentration range of 100 to 1000µg/ml. Thiourea as standard inhibitor. For urease inhibition assays after addition of 10ml of phosphate buffer to accurate weight of enzyme, sonication was performed, followed by centrifugation and absorbance of upper solution at 280nm. By using equation $A = \varepsilon bc$, where c is concentration of solution (mol/L), b is length of the UV cell and ε represents molar absorptivity, the concentration of initial urease solution was calculated.

After proper dilution, the concentration of enzyme solution was adjusted to 2mg/ml. Reaction mixture comprising 1.2ml of phosphate buffer solution (10mM potassium phosphate, 10mM lithium chloride and 1m Methylenediamine tetraacetic acid, pH 8.2 at 37 °C), 0.2ml of urease enzyme solution, and 0.1ml of test compound were subjected to incubation. Urease activity is determined by measuring the ammonia released during the reaction by modified spectrophotometric method described by Weatherburn [36]. The concentration of compounds that inhibited the hydrolysis of substrate by 50% (IC_{50}) is determined through monitoring the inhibition effect of various concentrations of phytochemicals in the assay followed by kinetics studies. The extent of the enzymatic reaction shall be calculated based on the following equation:

$$I\% = 100–(T/ C*100)$$

Where I (%) is the inhibition of the enzyme, T (test) is the absorbance of the tested sample in the presence of enzyme C (control) is the absorbance of the solvent in the presence of enzyme.

Statistical Analysis: All the assays were done in triplicates to test the reproducibility of them. All results are presented as mean ± S.E. SPSS 15.0 (statistical software) was used for statistical analysis of results. The values of $p < 0.05$ were considered statistically significant. Correlations among data obtained were calculated using Pearson's coefficient (r).

RESULTS AND DISCUSSION:

Extraction Yield: Aqueous and methanol extracts were prepared of the selected flora to examine phytochemicals qualitatively and quantitatively, antioxidant activity and urease inhibitory activity. The yield of extract obtained from 10g of dry plant material was measured for each extract by formula % yield = weight of extract obtained / weight of powder taken × 100 (**Table 2**). The highest yield was obtained for *Hemidesmus indicus*.

TABLE 2: % YIELD OF THE SELECTED MEDICINAL PLANTS

Plant species	% yield	
	Aqueous extract	Methanol extract
Alstonia scholaris	5.77%	3.45%
Calotropis procera	9.16%	8.36%
Rauvolfia serpentine	5.81%	2.92%
Hemidesmus indicus	17.85%	19.56%
Calotropis gigantean	8.81%	7.25%

Preliminary Phytochemical Screening: The listed plants revealed the presence of various phytochemicals *viz.* flavonoids, phenols, saponins, alkaloids and tannins. Among all, *Calotropis procera* and *Calotropis gigantean* shows the maximum amount of flavonoids (**Table 3**).

TABLE 3: QUALITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOL EXTRACT OF SELECTED FLORA

Plant species	Flavonoids		Phenols		Saponins		Alkaloids		Tannins	
	Aq	Met	Aq	Met	Aq	Met	Aq	Met	Aq	Met
Alstonia scholaris	+	+	+	+	+	+	+	+	+	+
Calotropis procera	+++	+ +	+	+	+	+	+	+	+	+
Rauvolfia serpentine	+	+	+	+	+	+	+	+	+	+
Hemidesmus indicus	+	+	+	+	+	+	+	+	+	+
Calotropis gigantean	++	+ +	+	+	+	+	+	+	+	+

+++: highly present, ++: moderately present, +: Low,-: absent. Aq: Aqueous extract, Met: methanol extract.

Quantitative Phytochemical Screening in Plant Extracts: The phytochemicals like Phenols, flavonoids are groups of natural products with variable structure that are well known for their beneficial effects on health possessing significant antimicrobial, anti- inflammatory and antioxidant activities [37]. Therefore in the present study extracts were evaluated for the quantitative estimation. Total phenolic content varies from 10.62 ± 1.10mgGAE/g min *Rauvolfia serpentine* (aq) to 45.67 ± 1.34mgGAE/gm of extract in *Hemidesmus indicus* (met) (**Table 4**). The Flavonoid content varies from 14.43 ± 2.37mgRU/gm in *Alstonia scholaris* (aq) to 104.11 ± 0.68mgRU/g in *Calotropis procera* (aq). In present study, total tannin content varies from 9.20 ± 0.35mgGAE/gm in *Hemidesmus indicus* (aq) to 35.45 ± 0.00mgGAE/gm in *Rauwolfia serpentine* (met). The ascorbic acid content varies from 31.87 ± 0.42 mgGAE/gm in *Alstonia scholaris* (aq) to 178.23 ± 0.00mgGAE/gm in *Hemidesmus indicus* (met).

TABLE 4: QUANTITATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOL EXTRACT OF SELECTED FLORA

Plant Species	Phenolic content (mg GAE/g)		Flavonoid content(mg RU/g)		Tannin content (mg GAE/g)		Ascorbic acid	
	Aq	Met	Aq	Met	Aq	Met	Aq	Met
Alstonia scholaris	28.45 ±0.62	22.85 ±0.06	14.43 ±2.37	17.28 ±1.82	26.64 ±1.14	22.42 ±1.75	31.87 ±0.42	100.62 ±0.76
Calotropis procera	15.28 ±0.02	17.42 ±0.03	104.11 ±1.21	92.34 ±0.70	11.02 ±0.01	13.05 ±0.68	85.63 ±0.00	94.64 ±0.00
Rauvolfia serpentine	10.62 ±1.10	13.45 ±1.15	18.26 ±0.70	22.47 ±0.07	31.23 ±0.00	35.45 ±0.00	161.32 ±1.43	78.43 ±1.32
Hemidesmus indicus	32.23 ±0.68	45.67 ±1.34	78.29 ±0.08	26.28 ±0.09	9.20 ±0.35	17.41 ±0.54	178.23 ±0.00	76.34 ±0.00

Calotropis gigantean	19.26 ±1.24	15.58 ±0.73	97.34 ±1.23	87.43 ±0.00	10.23 ±0.00	14.28 ±0.00	97.18 ±1.32	101.13 ±1.24

Aq: Aqueous extract, Met: Methanol extract.

***In vitro* Antioxidant Evaluation:** Antioxidants offer resistance against the oxidative stress by reducing the free radicals formation or scavenge the free radicals thus leads to escape from the damage caused by reactive oxygen species on the health of different organisms. To determine the efficacy of plant extracts as natural antioxidants, a number of *in vitro* methods have been developed in which antioxidant compounds can deactivate free radicals by mechanisms like HAT (Hydrogen atom transfer), SET (Single electron transfer), combination of HAT and SET and other standardised assays.

HAT based methods measure the classical ability of an antioxidant to quench the free radicals by hydrogen donation and SET based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound including metals, carbonyls and radicals. Since not a single assay can lead to the evaluation of the total antioxidant capacity of a compound therefore in the present study different assay are used. β- carotene bleaching assay is HAT based, FRAP is SET based, DPPH is based on the combination of both and chelating effect on ferrous ions is other standardised method [38].

In the present study, *Rauvolfia serpentine* shows the maximum scavenging activity of 55.64 + 0.40% in case of DPPH scavenging assay. However the antioxidant evaluation by metal chelating effect, antioxidant activity ranges from 22.48 + 0.00% to 62.70 + 0.20%. In case of FRAP assay, antioxidant activity ranges from 14.60 + 0.40 to 42.6 + 0.26%. *Calotropis procera* and *Calotropis gigantean* shows maximum antioxidant activity by β- carotene bleaching assay (**Fig. 1, 2, 3, 4**).

- DPPH, L-Ascorbic acid as standard: 78.26%
- Chelating effect on ferrous ions, EDTA as standard: 72.8%
- FRAP assay, Ferrous sulphate as standard.
- β - carotene bleaching method, Butylated hydroxytoluene (BHT) as standard: 85.24%.

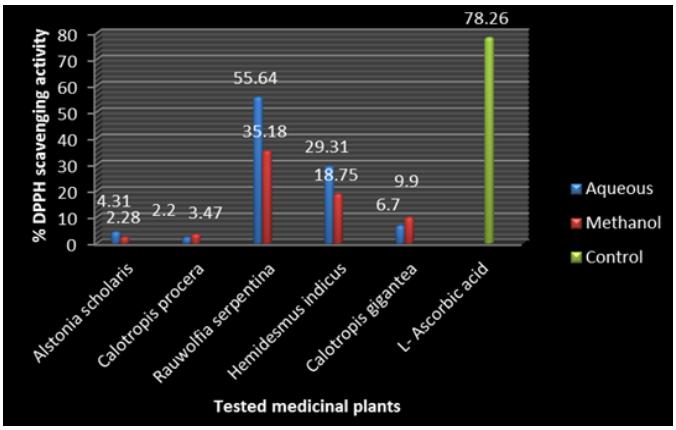

FIG. 1: SCAVENGING ACTIVITY (%) OF DIFFERENT PLANT EXTRACTS ON DPPH RADICALS

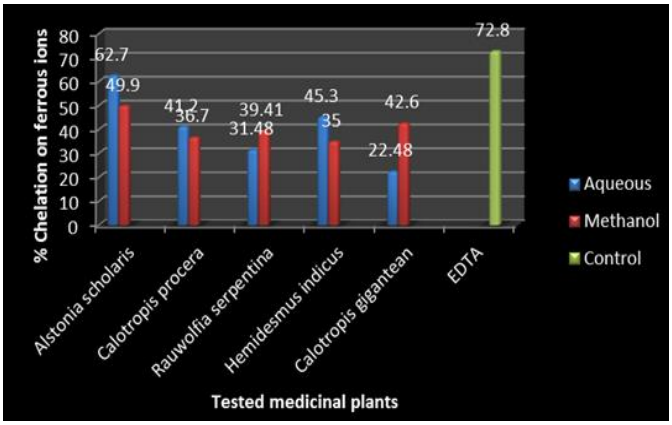

FIG. 2: FERROUS ION CHELATING ACTIVITY (%) OF VARIOUS EXTRACTS

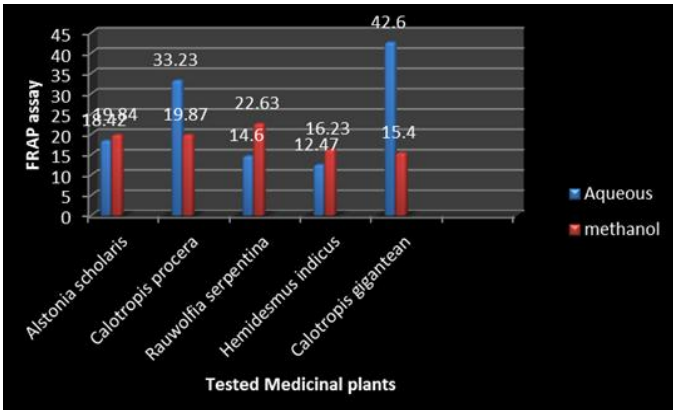

FIG. 3: FERRIC REDUCING ANTIOXIDANT CAPACITY (%) OF VARIOUS PLANT EXTRACTS

Urease Inhibition Studies: Urease is an enzyme that catalyses the hydrolysis of urea to ammonia and carbamate. This reaction leads to the significant increase in the pH and also has negative effects on human health. Although there are number of synthetic urease inhibitors available in

the market but due to their high cost and toxic nature, urease inhibitors from natural sources are highly recommended. In the present study, urease inhibition activity varies from 7.86 ± 0.67% inhibition to 40.48 + 0.00% inhibition in different plants. The urease inhibitory activities of the tested medicinal plants in descending order are as follows: *Calotropis gigantean* > *Calotropis procera* > *Alstonia scholaris* > *Hemidesmus indicus* > *Rauvolfia serpentine* (**Table 5**).

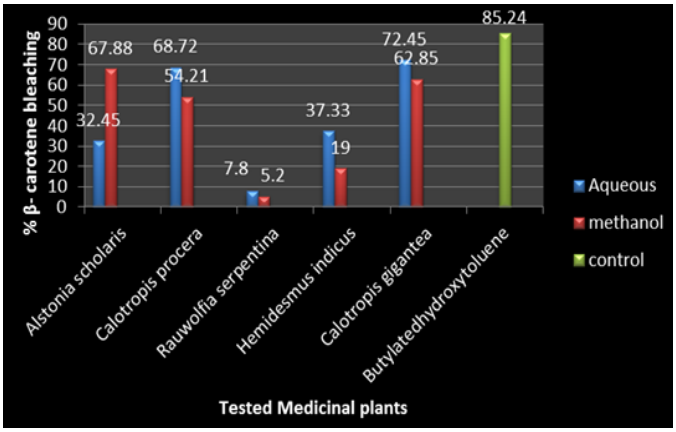

FIG. 4: ANTIOXIDANT ACTIVITY (%) OF VARIOUS PLANT EXTRACTS BY β- CAROTENE BLEACHING ASSAY

TABLE 5: UREASE INHIBITORY ACTIVITY OF TESTED PLANT EXTRACTS AT A CONCENTRATION 1mg/ml

Plant species	% inhibition*	IC_{50} mg/ml
Alstonia scholaris		
Aq	23.41±0.02	1.02
Met	25.88±0.03	0.86
Calotropis procera		
Aq	39.27±0.08	1.28
Met	39.68±0.08	1.64
Rauvolfia serpentine		
Aq	7.86±0.67	2.39
Met	8.30±0.34	2.75
Hemidesmus indicus		
Aq	12.81±0.03	-
Met	24.41±0.06	2.77
Calotropis gigantean		
Aq	40.48±0.00	1.34
Met	40.23±0.02	3.57

*Values are expressed as mean ± SD of triplicate experiments

Thiourea taken as standard in case of urease inhibitory profile and % inhibition = 71.47.

Aq: aqueous extract; Met: methanol extract.

In this present study, *Calotropis procera* and *Calotropis gigantean* shows the maximum amount of flavonoid compounds and the maximum antioxidant activity evaluated by the β - carotene bleaching assay. Tarik *et al.*, evaluated a similar pattern of positive correlation between flavonoid content and antioxidant activity by β - carotene assay [39]. Cao *et al.*, also indicated antioxidant behaviour of flavanoids [40]. The two plants, also exhibited the efficient urease inhibitory potential. Bina *et al.*, have also reported urease inhibitory potential of flavonoids of *Lawsonia alba* [41]. Flavonoid content and FRAP seems to have a raw positive correlation among all. The compound seems to provide some synergistic effect. The literature has also indicated positive correlation between two along with other factors [42].

The present assignment also indicated no correlation of Total phenol constituents and DPPH studies as already hinted by Hesam *et al.*, and Rafat *et al.*, [43, 44]. Although literature have also strongly supported positive correlation [45]. To potentiate the use of the *Calotropis procera* and *Calotropis gigantean* leaves extract as antioxidant and as remarkable urease inhibitor, correlation values were calculated between flavonoid and antioxidant activity by β- carotene bleaching assay, between flavonoid content and antioxidant potential by FRAP assay as well as between flavonoid content and urease inhibitory activity. The value of the r^2 shows the remarkable correlation between them respectively (**Table 6, 7** and **8**).

TABLE 6: r^2 VALUE REPRESENTING CORRELATION BETWEEN TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY EVALUATED BY β-CAROTENE AND LINOLEIC ACID ASSAY OF TESTED MEDICINAL PLANT EXTRACTS

Plant species	Aqueous extract	Methanol extract
Alstonia scholaris	0.9231	0.9273
Calotropis procera	0.9534	0.9657
Rauvolfia serpentine	0.2690	0.5460
Hemidesmus indicus	0.8673	0.7692
Calotropis gigantean	0.9476	0.9732

TABLE 7: r^2 VALUE REPRESENTING CORRELATION BETWEEN TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY EVALUATED BY FRAP ASSAY OF TESTED MEDICINAL PLANT EXTRACTS

Plant species	Aqueous extract	Methanol extract
Alstonia scholaris	0.9686	0.7933
Calotropis procera	0.9879	0.9603
Rauvolfia serpentine	0.8970	0.9998
Hemidesmus indicus	0.2187	0.9787
Calotropis gigantean	0.9421	0.9894

TABLE 8: r^2 VALUE REPRESENTING CORRELATION BETWEEN TOTAL FLAVONOID CONTENT AND UREASE INHIBITORY ACTIVITY OF TESTED MEDICINAL PLANT EXTRACTS

Plant species	Aqueous extract	Methanol extract
Alstonia scholaris	0.6865	0.7412
Calotropis procera	0.9627	0.9814
Rauvolfia serpentine	0.7367	0.6439
Hemidesmus indicus	0.9342	0.0335
Calotropis gigantean	0.9921	0.9541

CONCLUSION: The current study reveals a strong likelihood of correlationship between flavonoid content, β- carotene antioxidant potential and the urease inhibitory potential activity. The study also indicates availability of some phytochemicals responsible for urease inhibitory potential of the Genus Calotropis. The work validates traditional application of Calotropis as antioxidative and anti-ulcer. Further research towards genus could provide some chemically interesting and biologically active urease inhibitors.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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