The Pharma Innovation Journal 2018; 7(9): 270-276

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.03**
TPI 2018; 7(9): 270-276

www.thepharmajournal.com
Received: 15-07-2018
Accepted: 16-08-2018

**Neha Sikri**
Department of Biotechnology, Kurukshetra University, Kurukshetra, Haryana, India

**Sunita Dalal**
Department of Biotechnology, Kurukshetra University, Kurukshetra, Haryana, India

**Correspondence**
**Sunita Dalal**
Department of Biotechnology, Kurukshetra University, Kurukshetra, Haryana, India

# Plants of Acanthaceae family: Phenolic composition, enzyme inhibitory and antioxidant activities

**Neha Sikri and Sunita Dalal**

**Abstract**
The plants of Acanthaceae family traditionally been used as significant source of therapeutic preparation. For the treatment of various diseases like gastrointestinal tract & hepato-protective disorders, inflammatory diseases, fevers and snake poisoning as well. In the present study we have examined the antioxidant and urease inhibitory potential of aqueous and methanol extracts of leaves of two plants of Acanthaceae family viz. *Andrographis paniculata* and *Justicia adhatoda*. Different analysis revealed that the aqueous extract of *Andrographis paniculata* leaves not only exhibited significant antioxidant potential in different assays performed but also exhibited considerable urease inhibitory potential(45.32%) as compared to the standard inhibitor, thiourea exhibited percent inhibition 71.47%. Quantitative evaluation of phytochemicals also revealed that the aqueous extracts of *Andrographis paniculata* leaves possessed the high flavonoid and phenolic content (75.67 mgGAE/gm and 85.66 mgGAE/gm) respectively. This study validates that *Andrographis paniculata* leaves, part of various therapeutic preparations for many years, can be used for the treatment of gastrointestinal disorders, stomach related disorders and other urease related infections as well.

**Keywords:** Acanthaceae, urease inhibitory, thiourea, *Andrographis paniculata*

## 1. Introduction
Number of infections like gastro duodenal infections, hepatic coma, urothliasis, ammonia encephalopathy, gastric and peptic ulcers and many more are mainly associated with ureolytic activity of bacterial and fungal pathogens such as *Helicobacter pylori, Clostridium perforigens, klebsiella pneumonia, proteus mirabilis* and fungal species *Cryptococcus neoformans (a basidiomycete) and Coccidioides posadasii (an ascomycete)* [1-3].Out of these, one of the significant bacterial pathogens, *H. pylori* is able to develop infection in stomach due to the hydrolysis of urea by enzyme urease that provide ammonia and carbon dioxide which enable *H. pyroli* to raise pH of its environment,[2, 4, 5] it leads to its survivability in human stomach. *H. pylori* infection also leads to the gastric mucosal injury [6]. The urease activity associated with other pathogens leads to stone formation and other urinary tract infections. However eradication of these pathogens can be done by two or more antibiotics and a proton pump inhibitor [7]. To fight the infection the combination can be given with Bismuth salt (a metal) that have antiurease properties [8-11]. But the treatment efficacy can be reduced by acquisition of resistance by the bacterial pathogen to antibiotics [12]. So there is a need for the designing of new therapy for eradication of infections caused by urease enzyme. Till now, different organic compounds, five and six member heterocyclic compounds, barbituric acid derivatives and also the organophosphorus compounds do act as urease inhibitors [13]. But features like low hydrolytic stability and toxicity, renders their use. So, the natural sources can act as better alternatives.

Since the ancient times, people of different cultures and regions are using the medicinal plants traditionally due to their therapeutic properties [14-16]. So, the medicinal plants can prove to be an alternative source for ingredients that can be used in the therapeutic preparations and this can also be termed as alternative medicine.

Various metabolic processes like respiration, cell mediated immune function and many more leads to the continuous production of ROS (reactive oxygen species) and free radicals. These reactive species are also generated by environmental pollutants, smoking and pesticides also [17]. These free radicals could not be able to cause the deleterious effects on the body due to the antioxidant mechanisms present in the body in the balanced way [17]. But sometimes the imbalance generated in the cell system leads to the instability of the reactive species as they have lone pairs in their outer shell. In order to attain the stability they then attack the biological

molecules of the living cells such as lipids, proteins, DNA & RNA [18]. This bimolecular degradation associated with various diseases like cardiovascular diseases, inflammatory diseases, liver diseases, cancer, AIDS, premature aging and neurodegenerative diseases due to a phenomenon lipid per oxidation [18, 19]. Generally, the antioxidants functions to deter the deleterious effects of the free radicals on human body [20]. Till date, synthetic antioxidants like BHT, BHQ are used as drugs but these possess the potential risk. So, there is need for some natural antioxidants with no side effects.

Acanthaceae, a family of dicotyledenous flowering plants spread all over the world. Out of the 4300 species and 346 genera's, With 600 species Justicia is the largest genus of the family [21]. 'Acan' word in the Acanthaceae means 'it washes' because leaves of the plants of this family contains oil and it is used in laundry as well [22]. The different plants of this family possesses compelling therapeutic uses due to the presence of bioactive compounds like alkaloids, terpenoids, phenols, cardiac glycosides and different proteins also. Different plant extracts contains chemicals like acetaldehyde, cyclopentane and many more so they are used in pharmaceutical and cosmetic industries as well [23]. Paste of the leaves of the plants like *Justicia flava* and *Justicia betonica* are used for treatment of snake bites, dry cough, ulcers, diarrohea and eye infections [24]. Acanthaceae family members like *Hygrophila spinosa*, *Barleria prionitis* and *Adhatoda vasica* are traditionally used as antipyretic, anti-asthmatic and in respiratory diseases as well. In the present study, leaf extracts of two plants of Acanthaceae family viz. *Andrographis paniculata* and *Justicia adhatoda* were evaluated to validate their use in therapeutic preparations. Out of these, *Andrographis paniculata* known as 'King of Bitters' use in folk medicine from ancient times. Traditionally, it is being used as antimicrobial, anti- diabetic, sex hormone modulatory and also as anti-insecticides [25, 26]. The other member used in the study was of Justicia species viz. *Justicia adhatoda* used for swellings, pneumonia malaria, cold and cough [27].

The purpose of this study is to evaluate the *in vitro* antioxidant and urease inhibitory activities of methanol and water extracts of *Andrographis paniculata* and *Justicia adhatoda* leaves. Antioxidant activity was analyzed by using DPPH, metal chelating, β-carotene bleaching assay and reducing power assays. Additionally, the relationship between the phytochemical composition and biological activity also discussed.

## 2. Materials and methods

### 2.1 Chemicals

Urease type 1X (specific activity: 50,000-100000units/g) from *Canavalia ensiformis* (L.) DC. (Fabaceae) commonly known as Jack-Bean was purchased from Sigma Aldrich (St. Louis, MO). 2, 2´diphenyl1picrylhydrazyl, 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.

### 2.2. 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˚48'52.128" 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.

### 2.3 Qualitative and quantitative phytochemical screening

- **Qualitative phytochemical screening**

Both extracts of the selected plants were evaluated for the screening of the various phytochemicals using standard phytochemical procedures [28].

- **Estimation of total phenolic content**

The content of total phenols was determined according to the Folin-Ciocalteu's method [29]. 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 90min 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 *et al*., 2000 [30]. 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 [31]. 7.5ml of distilled water was added in0.1ml 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 up to 10ml with distilled water. Mixture was kept at room temperature for 30 minutes and absorbance was taken at 725 nm. 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, 1953 [32]. Briefly, ascorbic acid was oxidized by activated charcoals to yield dehydroascorbic acid, which further react with 2,4-dinitrophenylhydrazine 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.

### 2.4 *In vitro* antioxidant evaluation

- **2, 2diphenyl1picrylhydrazyl scavenging assay**

The ability of the plant extract to scavenge DPPH free radicals was assessed by the method described by Mensor *et al*., 2001[33]. The L-ascorbic acid was used as a positive control. The reaction mixture was prepared containing 300 µl of extract of varying concentrations (1-100 µg/ml) and 2 ml of DPPH (0.1 mM in methanol). The reaction mixture was then placed in the cuvette holder of the spectrophotometer and the absorbance measure at 517 nm 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 *et al*. (1994) [34]. The extracts (0.25 ml) were mixed with 1.75 ml of methanol and 0.25 ml of 250 mM $FeCl_2$. This was followed by the addition of 0.25 ml of 2 mM ferrozine, and kept at room temperature for 10 min. before determining the absorbance of the mixture at 562 nm. 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 *et al*., 1996 [35]. 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 $^0$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 $^0$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, 1971 [36]. BHT was used for comparison. 2 mg of β-carotene dissolved in chloroform (10 ml) was pipette into a small, round-bottom flask. After the chloroform was removed at 40 °C under vacuum, 100 µl of linoleic acid, 400 mg of tween 20 and 100 ml of oxygenated water was added to the flask followed by vigorous mechanical stirring. 5 ml of the above prepared emulsion was transferred to a series of test tubes containing 200 µl of extracts of various concentrations. As soon as the emulsion was added to each tube, the zero time absorbance is measured at 470 nm 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 470 nm. 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.

### 2.5 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 10 ml of phosphate buffer to accurate weight of enzyme, sonication was performed, followed by centrifugation and absorbance of upper solution at 280 nm. By using equation A= εbc, 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 2 mg/ml. Reaction mixture comprising 1.2 ml of phosphate buffer solution (10mM potassium phosphate, 10mM lithium chloride and 1mMethylenediaminetetraacetic acid, pH 8.2 at 37 °C), 0.2 ml of urease enzyme solution, and 0.1 ml 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, 1967 [37]. The concentration of compounds that inhibited the hydrolysis of substrate by 50% (IC50) 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.

### 2.6 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).

## 3. Results and Discussion

**3.1. Extraction Yield:** Aqueous and methanol extracts were prepared of the selected medicinal herb 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 1).

**Table 1:**% yield of studied plant extracts

| Plant Species | % Yield | |
|---|---|---|
| | **Aqueous extract** | **Methanol Extract** |
| *Andrographis paniculata* | 9.76% | 7.31% |
| *Justicia adhatoda* | 8.79% | 7.68% |

### 3.2. Qualtitative & quantitative phytochemical screening

- **Qualtitative Phytochemical screening**

The studied plant extracts revealed the presence of various phytochemicals viz. phenols, flavonoids, saponins, alkaloids and tannins. Out of various phytochemicals analyzed, in

*Andrographis paniculata* leaf extracts flavonoids and phenols and in *Justicia adhatoda* tannins, alkaloids and flavonoids are present in significant quantity (Table 2).

**Table 2:** Qualitative phytochemical screening of Aqueous and methanol extract

| Plant species | Flavonoids | | Phenols | | Saponins | | Alkaloids | | Tannins | |
|---|---|---|---|---|---|---|---|---|---|---|
| | aq | met | aq | met | aq | met | aq | met | aq | met |
| *Andrographis paniculata* | ++ | +++ | ++ | +++ | + | + | + | + | + | + |
| *Justicia adhatoda* | +++ | +++ | + | + | + | + | ++ | ++ | ++ | +++ |

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

- **Quantitative phytochemical estimation**

Total phenolic content varies from 18.91±0.10mgGAE/gm in methanol extract of *Justicia adhatoda* to 87.66±0.34mg GAE/gm of extract in aqueous extract of *Andrographis paniculata* (Table. 3). The Flavonoid content varies from 51.04±0.39 mgRU/gm in aqueous extract of *Justicia adhatoda* to 95.67±1.08mgRU/g in aqueous extract of *Andrographis paniculata*. Current study evaluated that total tannin content varies from 20.04±0.98 mg GAE/gm in methanol extract of *Justicia adhatoda* to 45.70±1.75mg GAE/gm in aqueous extract of *Andrographis paniculata*. The ascorbic acid content varies from 12.54±1.02 mg GAE/gm in methanol extract of *Justicia adhatoda* to 47.98±1.42 mgGAE/gm in aqueous extract of *Andrographis paniculata*.

**Table 3:** Quantitative estimation of phytochemicals of aqueous and methanol extract

| Plant species | Phenolic (mg GAE/g) | | Flavonoid (mg RU/g) | | Tannin (mg GAE/g) | | Ascorbic acid | |
|---|---|---|---|---|---|---|---|---|
| | Aq | met | Aq | met | Aq | met | Aq | met |
| *Andrographis paniculata* | 87.66 ±0.34 | 73.33± 0.98 | 95.67 ±1.08 | 82.45 ±1.34 | 45.47 ±1.08 | 32.48 ±0.76 | 47.32 ±0.45 | 21.98 ±1.57 |
| *Justicia adhatoda* | 26.36 ±1.74 | 18.91 ±0.65 | 51.04 ±1.09 | 74.26 ±2.65 | 24.36 ±1.98 | 20.04 ±0.67 | 25.67 ±0.21 | 12.54 ±0.45 |

Aq: Aqueous extract, Met: Methanol extract.

### 3.3 Evaluation of Antioxidant activity

In the present study, a total of four methods including, DPPH free radical-scavenging activity, ferric reducing/antioxidant power (FRAP)- reducing assay, Chelating effect on ferrous ions and β- carotene bleaching assay were studied. In order to become stable diamagnetic molecules, they have the ability to release hydrogen radical or electron to DPPH and this phenomenon is called as antioxidant activity. So, the reason for the higher antioxidant activity of the above-mentioned extracts with DPPH might be the same. However, antioxidant activity of the extracts is also well explained by FRAP, chelating capacity and bleaching assay. Antioxidant activity of the compounds is summarized in figures1, 2, 3&4. However, *Andrographis paniculata* (aq) shows the maximum scavenging activity of 69.70±0.40% in case of DPPH scavenging assay. The antioxidant evaluation by metal chelating effect, antioxidant activity ranges from 32.14±0.09%in *Andrographis paniculata* (met) to 45.62±0.20% in *Andrographis paniculata* (aq). In case of FRAP assay, antioxidant activity ranges from 7.80±0.40 in *Andrographis paniculata* (met) to 17.44±0.26% in *Justicia adhatoda* (met). However, the maximum antioxidant activity by β- carotene bleaching assay by Justicia adhatoda (aq).

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, Butylatedhydroxytoluene (BHT) as standard: 85.24%.

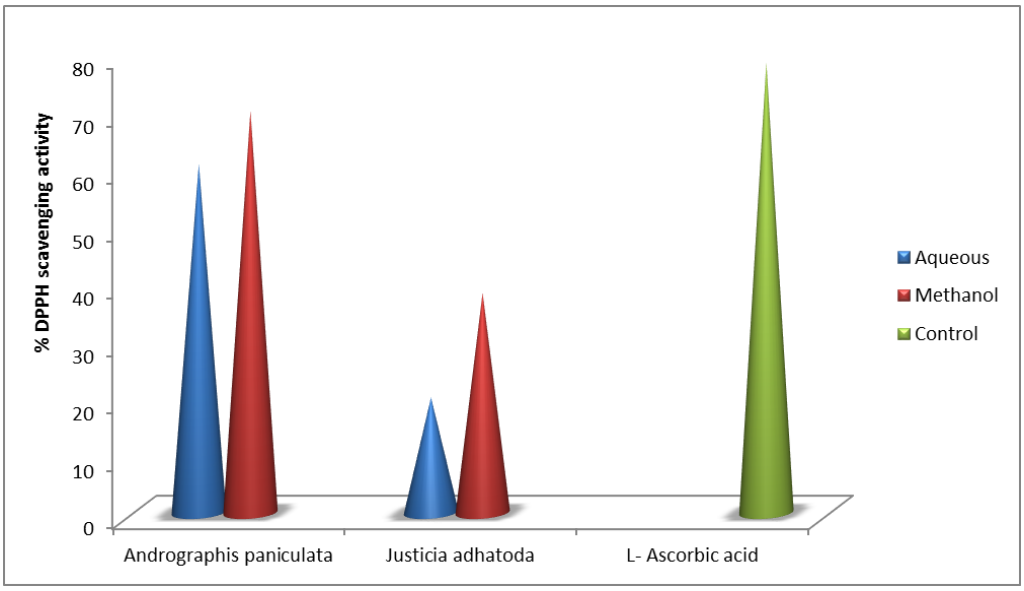


**Fig 1:** Scavenging activity (%) of different plant extracts on DPPH radicals

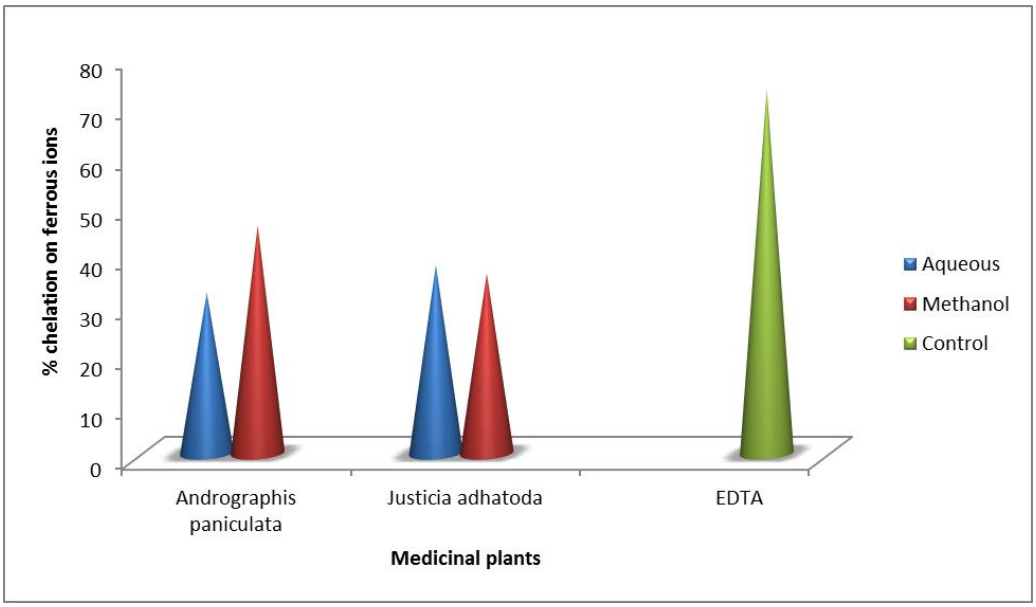


**Fig 2:** Ferrous ion chelating activity (%) of various extracts

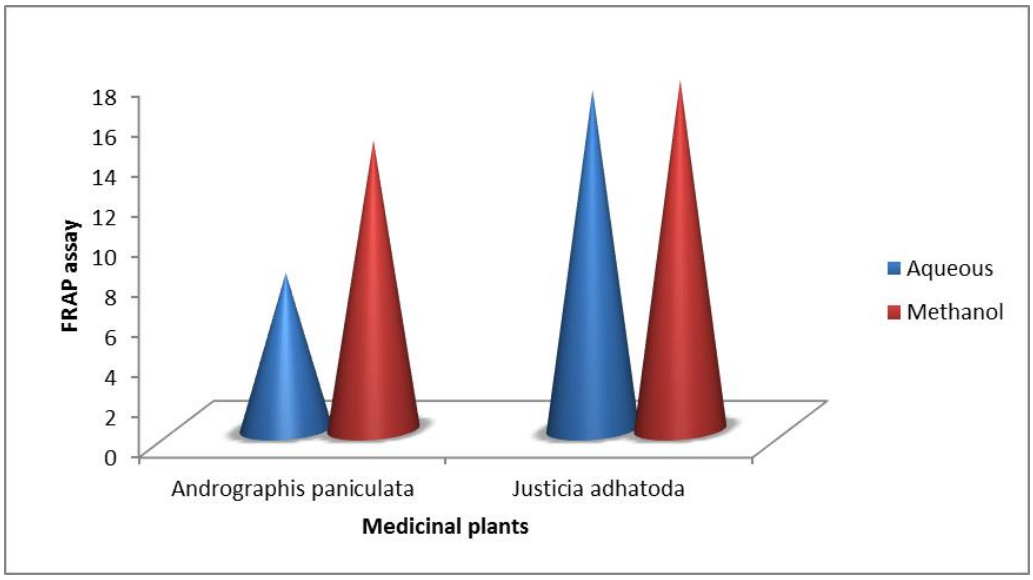


**Fig 3:** Ferric reducing antioxidant capacity (%) of various plant extracts

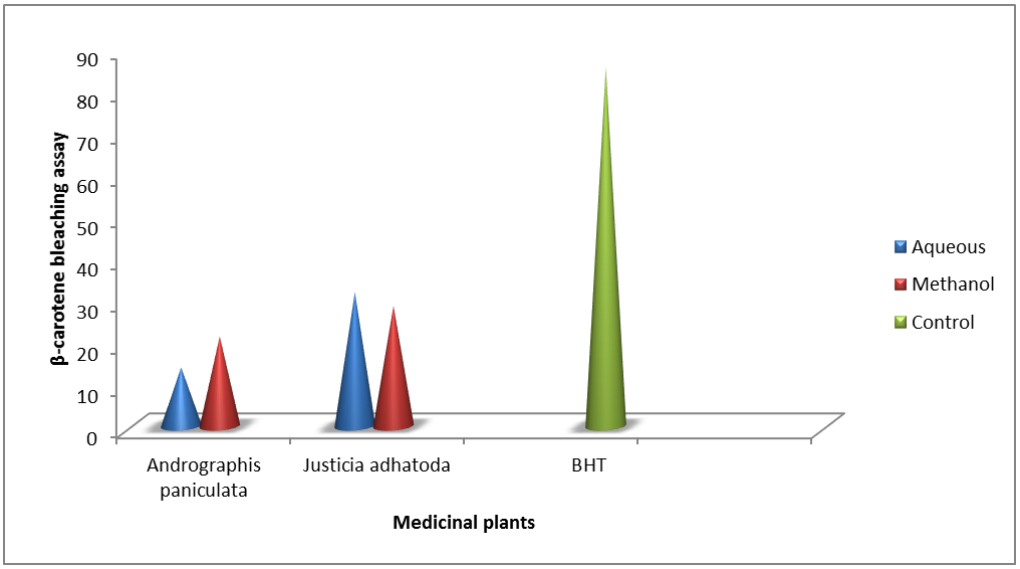


**Fig 4:** Antioxidant activity (%) of various plant extracts by β- carotene bleaching assay

### 3.4 Urease inhibition studies

As in the occurrence of various pathological conditions such as gastric and peptic ulcers, stomach cancers, urolithiasis and in many more, urease enzyme plays an important role. However, there are numbers of inhibitors synthetically prepared but due to their toxic and unstable nature; there is a need to discover natural and safe ones. In the present study, urease inhibitory potential of aqueous and methanol extract of two plants viz, *Andrographis paniculata* and *Justicia adhatoda* was studied (Table 4). The urease inhibitory potential varies from 19.21±0.09 to 45.23±0.02%.

**Table 4:** Urease inhibitory activity of tested plant extracts at a concentration 1mg/ml.

| Plant species | % inhibition | $IC_{50}$mg/ml |
|---|---|---|
| *Andrographis paniculata* | | |
| Aq | 32.23±0.02 | 1.02 |
| Met | 45.21±0.03 | 0.86 |
| *Justicia adhatoda* | | |
| Aq | 15.21±0.08 | 1.28 |
| Met | 21.24±0.34 | 1.64 |

Out of the two plants of Acanthaceae family studied, *Andrographis paniculata* posses high amount of flavonoids, phenolic content and other phytochemicals, good antioxidant potential as well as significant urease inhibition activity. There are many reports in the literature that show the strong correlation between flavonoids and antioxidant capacity exhibited by different medicinal plant extracts [38]. In the present study, *Andrographis paniculata* extracts exhibited high flavonoid content also possesses strong antioxidant capacity. Lalitha *et al.*, [39] and Malahubban *et al*.,[40] revealed that methanol extract of the *Andrographis paniculata* leaves possesses higher antioxidant capacity as compared to the aqueous extract which validates the present study. Literature revealed Andrographolide (C20H30O5), a diterpenoid lactone and the effective constituent of *Andrographis paniculata* act as competitive inhibitor of urease enzyme which can be correlated with significant urease inhibition activity of one of the extracts in our study [41].

## 4. Conclusion

The present study reveals a strong likelihood of correlationship between flavonoid content and antioxidant potential. The study verifies availability of some phytochemicals that could be responsible for the significant urease inhibitory potential of the *Andrographis paniculata*. The work validates traditional application of *Andrographis paniculata* as antioxidative and anti-ulcer. Further research towards the identification of the compound responsible need to be carried out by bioassay guided studies.

## 5. Acknowledgements

The authors greatly acknowledge the Haryana State Council for Science and Technology (HSCST) for financial support for the project entitled "Urease Inhibitors from Medicinal plants of Haryana in context to agricultural soil protection and therapy against clinically significant virulence factor" (Endst. No. HSCST/R&D/2015/ 2383).

## 6. References

1. Weeks D, Eskandari S, Scott DR, Sachs G. A H+ - gated urea channel: the link between Helicobacter pylori urease and gastric colonization. Science. 2000; 287:482-485.
2. Burne RA, Chen YM. Bacterial ureases in infectious diseases. Microb Infect. 2000; 2:533-542.
3. Rutherford JC. The emerging role of urease as a general microbial virulence factor. PLoS Pathog. 2014; 10:e1004062.
4. Mollenhauer-Rektorschek M, Hanauer G, Sacks G, Melchers K. Expression of UreI is required for intragastric transit and colonization of gerbil gastric mucosa by Helicobacter pylori. Res Microbiol. 2002; 153:659-666.
5. Skouloubris S, Thiberge JM, Labigne A, De Reuse H. The helicobacter pylori UreI protein is not involved in urease activity but is essential for bacterial survival *in vivo*. Infect Immun. 1998; 66:4517-4521.
6. Smoot DT, Mobley HL, Chippendale GR, Lewison JF, Resau JH. Helicobacter pylori urease activity is toxic to human gastric epithelial cells. Infect Immun. 1990; 58:1992-1994.
7. Hung IF, Chan P, Leung S, Chan FS, Hsu A, But D *et al*. Clarithromycin-amoxycillin containing triple therapy: a valid empirical first-line treatment for Helicobacter pylori eradication in Hong Kong? Helicobacter. 2009; 14(6):505-511.
8. Follmer C. Ureases as a target for the treatment of gastric and urinary infections. J Clin Pathol. 2010; 63:424-430.
9. Mégraud F. The challenge of *Helicobacter pylori* resistance to antibiotics: the comeback of bismuth-based quadruple therapy. Therap Adv Gastroenterol, 2012, 103-109.
10. Zhang L, Mulrooney SB, Leung AFK, Zeng Y, Ko BBC, Hausinger RP *et al.* Inhibition of urease by bismuth (III): Implications for the mechanism of action of bismuth drugs Biometals. 2006 ; 19:503-511.
11. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D *et al*. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report Gut. 2007; 56:772-781.
12. Glupczynski Y. Antimicrobial resistance in Helicobacter pylori: a global overview. Acta Gastroenterol Belg. 1998; 61:357-366.
13. Rego YF, Queiroz MP, Brito OT, Carvalho PG, Queiroz VTD, Fátima AD *et al*. A review on the development of urease inhibitors as antimicrobial agents against pathogenic bacteria, Journal of Advanced Research. 2018; 13:69-100.
14. Bahmani M, Banihabib EK. Comparative assessment of the anti-annelida (*Limnatis nilotica*) activity of nicotine with niclosamide. Global Veterinaria. 2013; 10:153-7.
15. Amirmohammadi M, Khajoenia SH, Bahmani M, Rafieian-Kopaei M, Eftekhari Z, Qorbani M. *In vivo* evaluation of antiparasitic effects of *Artemisia abrotanum* and *Salvia officinalis* extracts on *Syphacia obvelata*, *Aspiculoris tetrapetra* and *Hymenolepis nana* parasites. Asian Pac J Trop Dis. 2014; 4:250-4.
16. Bahmani M, Eftekhari Z. An ethnoveterinary study of medicinal plants in treatment of diseases and syndromes of herd dog in southern regions of Ilam province, Iran. Comp Clin Path. 2012; 22:403-7.
17. Masoko P. Eloff JN. Screening of twenty-four South African Combretum and six Terminalia species for antioxidant activities. African Journal of Traditional, Complementary and Alternative Medicines. 2007; 4(2):231-239.
18. Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR.

Antioxidant activity and free radical scavenging potential of *Pithecellobium dulce* Benth seed extracts. Free Radical and Antioxidants. 2011; 2(2):37-43.
19. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: A review. Reproductive Biology and Endocrinology 2012; 10(49).
20. Laguerre M, Giraldo LJL, Lecomte J, Figueroa-Espinoza MC, Barea B, Weiss J *et al*. Chain length affects antioxidant properties of chlorogenate esters in emulsion: the cutoff theory behind the polar paradox. J. Agric. Food Chem. 2009; 57:11335-11342.
21. Durkee L. Flora Costaricensis: family. Acanthaceae. Field Bot. 1996; l(18):1-87.
22. Mabberley DJ. Mabberley's plant-book: a portable dictionary of plants, their classifications and uses. Ed 3, Cambridge University Press, 2008.
23. Doss A, Rukhshana MS, Rani KP. Identification and properties of *Asteracantha longifolia* (L.) Nees by GC-MS analysis. J Adv Med Life Sci. 2017; 5(1):1-6.
24. Jeruto P, Lukhoba C, Ouma G, Otieno D, Mutai C. An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. J Ethnopharmacol. 2008; 116(2):370-376.
25. Mishra SK, Sangwan NS, Sangwan RS. *Andrographis paniculata* (Kalmegh): A review. Pharmacognosy Rev. 2007; 1:283-298.
26. Khare CP. *Andrographis paniculata*. Indian medicinal plants, an Illustrated Dictionary. Springer. New Delhi, India, 2007, 49-50.
27. Sharma M, Ahmad J, Hussain A *et al*. Folklore medicinal plants of Mewat (Gurgaon district), Haryana, India. Int J pharm. 1992; 30(2):129-134.
28. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, Nirali Prakashan Edition 2nd, 2000, 14956.
29. Singleton VL, Orthofer R, Lamuela RRM. Analysis of total phenols and other oxidation substrates and means of folin Ciocalteu reagent. Methods Enzymology. 1999; 299:152-178.
30. Quettier DC, Gressier B, Vasseur J. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. Journal of Ethnopharmacology. 2000; 72:35-42.
31. Singh R, Verma PK, Singh G. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. J Intercult Ethnopharmacol. 2012; 1(2):101-104.
32. Roe JH, Keuther CE. The determination of ascorbic acid in whole blood and wine through 2, 4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 1953; 147(5):399-405.
33. Mensor LL, Menzes FS. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research. 2001; 15:127-130.
34. Dinis TCP, Madeira VMC. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical sacavangers. Arch. Biochem. Biophysics 1994; 315:161-169.
35. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. Analytical Biochemistry. 1996; 239:70-76.
36. Miller HE. A Simplified method for the evaluation of antioxidants. J Am. Oil Chem. Soc. 1971; 48:91-95.
37. Weatherburn MW. Phenol- hypochlorite reaction for determination of ammonia. Analytical Chemistry. 1967; 39:971-974.
38. Kar A. Pharmaocgnosy and Pharmacobiotechnology. Revised-Expanded Second Edition. New Age International Limted Publishres. New Delhi, 2007, 332-600.
39. Lalitha G, Nazeema TH, 1sharmila I. Phytochemical screening and evaluation of antimicrobial activity, antioxidant activity, anticoagulant activity and fibrinolytic activity of leaves of *Andrographis paniculata* (leaf). Int j pharm bio sci 2015; 6(2):475- 484.
40. Malahubban M, Alimon AR, Sazili AQ, Fakurazi S, Zakry FA. Phytochemical analysis of *Andrographis paniculata* and *Orthosiphon stamineus* leaf extracts for their antibacterial and antioxidant potential. Trop Biomed 2013; 30(3):467-80.
41. Zhi-Zhun M, Xiu-Fen W, Xie Z, Ji-Yan S, Hai-Ming C, Yu-Hong L *et al*. Andrographolide sodium bisulphite-induced inactivation of urease: inhibitory potency, kinetics and mechanism. BMC Complementary and Alternative Medicine. 2015; 15:238.