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Original Article

# *In vitro* Antioxidant Activity and Preliminary Phytochemical Evaluation of Different Extracts of *Aerva javanica*

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**ABSTRACT**

**Background:** Ethno medicinal validation, together with the screening of phytochemical and biological activity, is an effective method for identifying new drugs from medicinal herbs. **Objectives:** The present study was aimed to investigate the phytochemical nature and antioxidant potential of the selected plant *Aerva javanica*. **Materials and Methods:** The plant material of *A. javanica* was collected, authenticated, leaf and stem parts were separated, shade dried, powdered separately in to coarse powder and extracted with ethanol by Soxhlet apparatus. Both extracts (leaves and stem) were subjected to phytochemical evaluation, total phenolic content estimation by Folin-Ciocalteau and the total flavonoids content estimation by using aluminium chloride colorimetric method. *In vitro* antioxidant evaluation of the leaves and stem extracts were done by different approaches such as DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, reducing power assay, superoxide and nitric oxide radical scavenging assays. **Results:** In the preliminary phytochemical analysis, both leaves and stem extract showed the presence of alkaloids, glycosides, tannins and phenolic compounds and flavonoids. The results showed the absence of carbohydrates, proteins and amino acids and sterols. The total phenolic content in the ethanol extract of *A. javanica* leaves and stems were found to be 891mg/g and 841mg/g of extract equivalent to gallic acid. The total flavonoids content in the ethanol extract of *A. javanica* leaves and stems were found to be 950mg/g and 900mg/g of extract equivalent to quercetin. The leaves extract showed maximum antioxidant activity comparing with stem extract in all the test approaches employed. **Conclusion:** The chromatographic and spectral studies and detailed pharmacological evaluation of the extracts may give more promising data.

**Keywords:** *Aerva javanica*, Preliminary phytochemical evaluation, Total phenolic estimation, Total flavonoid estimation, Antioxidant activity.

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## INTRODUCTION

Ethno medicinal validation, together with the screening of phytochemical and biological activity, is an effective method for identifying new drugs from medicinal herbs.[1] The genus Aerva is such a one. The Aerva species belongs to the family Amaranthaceae are hoary-tomentose herbs, up to 1m in height, commonly found in Africa and Asian countries such as Srilanka, Myanamar and Peninsular India. There are approximately 28 species in this genus.[2,3] The plant *Aerva javanica* is one among them. It was documented that the roots and flowers of *A. javanica* are effective in the management of kidney troubles and rheumatism. The decoction of the plant is used to get rid of swellings. Also, this plant is reported as demulcent, diuretic and anthelmintic. Seed of this plant is used for the treatment of headache. Powder of the plant is used to cure ulcers of domestic animals.[3,4] Based on this fact, the plant *A. javanica* is selected as the candidate for our research.

Oxidative stress, due to inner factors such as reactive oxygen species (ROS) viz., hydroxyl, nitric oxide, hypochlorite and superoxide anion radicals, hydrogen peroxide, singlet oxygen, etc., and extrinsic factors such as pollution, smoking, ionizing radiation, organic solvents, pesticides, etc.,[5] is the risk factors leads to numerous several chronic diseases such as asthma, inflammatory diabetes, cancers, atherosclerosis etc.,[6] Cells of mammals possesses enzymes such as superoxide dismutase, catalase or glutathione peroxidase as intracellular defences to protect the cells from the free radicals injury. Extraneous addition of antioxidants such as vitamins viz., A and E), minerals viz., Se and Zn, and proteins viz., transferrin, ceruloplasmin, albumin give added protection. Antioxidants have been used mainly in the food industry to lengthen the shelf life of food products particularly rich in polyunsaturated fats which are susceptible to oxidation readily and are a major cause of spoilage like quality deterioration, nutritional losses, off-flavour development and discolouration. Usage of artificial antioxidants, such as propyl gallate, butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary

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butyl hydroquinone have been prohibited in food industry due to their potential health risks and toxicity. Nowadays, the interest for searching antioxidants from natural sources has received much attention.[7] Hence the present study was carried out to investigate the phytochemical nature and antioxidant activity of the selected plant as a direction for further research.

## MATERIALS AND METHODS

### Collection and Identification of Plant

The plant material of *Aerva javanica* was collected from Kovai estate, Coimbatore, Tamil Nadu, India. The collected plant was identified and authenticated in the Botanical survey of India, The Southern Regional Centre., Coimbatore, Tamil Nadu (BSI/SRC/5/23/2013-14/Tech./1438).

### Powdering and Extraction of Plant Material

Powdering and extraction of collected plant material was done in reference with the previous literature.[8-12] The leaf and the stem was separated and dried in the shade separately for about two weeks, powdered individually in mechanical grinder and stored air tightly for further study. About 2kg of leaf and stem powder of *Aerva javanica* was extracted with ethanol by using Soxhlet apparatus assembly for 48h in five times, the extracts obtained were concentrated by simple distillation. Finally the crude extracts were stored in desiccators for further studies.

### Preliminary Phytochemical Analysis

The crude extract of leaves and stem of *Aerva javanica* was subjected to preliminary phytochemical analysis in accordance with the modified procedure in the published literatures.[8-11]

### Test for alkaloids

Mixture of little quantity crude extract, alcohol and dilute hydrochloric acid was shaken well, filtered, and the filtrate was used to detect the presence of alkaloids by the following tests.

#### *Mayer's test*

Little quantity of acidified filtrate and Mayer's reagent were mixed, shaken well and observed for the presence of creamy precipitate.

#### *Wagner's test*

Little quantity of acidified filtrate and Wagner's reagent were mixed and observed for the presence of reddish-brown precipitate.

#### *Hager's test*

Little quantity of acidified filtrate and Hager's reagent were mixed and observed for the presence of yellow precipitate.

#### *Dragendorff's test*

Little quantity of acidified filtrate and Dragendorff's reagent were mixed and observed for the presence of orange-red precipitate.

### Tests for glycosides

A pinch of crude extract was mixed with dilute hydrochloric acid and kept on a water bath for a few hours for hydrolysis and the hydrolysate obtained was used for the test for glycosides.

#### *Legal test*

Little quantity of hydrolysate was mixed with pyridine. To this mixture, little amount of freshly prepared sodium nitroprusside solution was added and made the mixture alkaline with sodium hydroxide solution and observed for the formation of pink colour.

#### *Baljet test*

Little quantity of hydrolysate was treated with little quantity of sodium picrate solution and observed for the formation of a yellow to orange colour.

### Tests for sterols

A little of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution for saponification. The saponified mixture was diluted and extracted with solvent ether, evaporated and the residue obtained was used for the tests for sterols.

#### *Liebermann – Burchard test*

The residue with little quantity of dry chloroform was mixed with little of acetic anhydride followed by the addition of concentrated sulphuric acid through the sides of the test tube and observed for the formation of green colour in the upper portion which changes to bluish violet.

#### *Salkowski test*

The mixture of residue with chloroform and concentrated sulphuric acid was observed for the red colour in the lower layer.

### Tests for phenolic compounds and tannins

#### *Ferric chloride test*

Little quantity of the extract with water and dilute ferric chloride solution (5%) was observed for the formation of blue colour

#### *Lead acetate test*

Little quantity of the extract with water and lead acetate solution (10%) was observed for the formation of bulky white precipitate.

### Tests for flavanones and flavonoids

#### *Aqueous sodium hydroxide test*

Little quantity of the extract was mixed with aqueous sodium hydroxide solution and observed for the formation of yellow colour.

#### *Ammonia test*

The filter paper wetted with alcoholic solution of the extract was exposed to ammonia vapour and observed for the formation of yellow colour.

#### *Shinoda test*

Little quantity of alcoholic extract was treated with magnesium or zinc and dilute hydrochloric acid and observed for the formation of orange-red or violet colour.

### Tests for carbohydrates

A little of ethanolic extract was mixed with water and filtered. The filtrate was used for the tests for carbohydrates.

#### *Molisch's test*

Little quantity of filtrate was treated with a few drops of Molisch's reagent and concentrated sulphuric acid was added through the side of the test tube without shaking and observed for the presence of violet ring at the junction of two solutions.

#### *Fehling's test*

Little quantity of filtrate was treated with Fehling's solution A and B (1ml) and boiled in a water bath and observed for the formation of a reddish precipitate.

#### *Benedict's test*

Little quantity of filtrate was treated with Benedict's reagent. Then the mixture was heated in a boiling water bath and observed for the formation of reddish precipitate.

## Tests for proteins and amino acids

### *Millon's test*

Mixture of extract and Millon's reagent was observed for the formation of white precipitate, which on warming turn into a red-coloured solution.

### *Ninhydrin test*

Mixture of extract with few drops of ninhydrin solution, heated on a water bath was observed for the presence of violet colour.

## Quantification of total phenolics and flavonoids

Based on the results of phytochemical screening, the leaves and stem extracts were subjected to the quantification of total phenolics and flavonoids content.

## Estimation of total phenolic content

Total phenolic content estimation was done by Folin-Ciocalteau assay method in accordance with the modified procedure.[13-15]

The extract or standard solution of Gallic acid (0.5ml) in different concentration (10, 20, 40, 60, 80 and 100µg/ml) were mixed with equal volume of Folin-Ciocalteau reagent. After 5min, 1ml of 20% sodium carbonate solution was added and the volume was made in to 10ml by distilled water, shaken well and kept the mixture at dark condition for 45min. Reagent blank using distilled water was also prepared. Absorbance of the mixture was measured against reagent blank at 750nm. The total phenolic content were expressed as milligrams of Gallic equivalents per gram extract. The total phenolic content in the test extracts was expressed as milligram of gallic acid equivalent (mg GAE/g extract) by using the standards curve.

## Estimation of total flavonoids content

The total flavonoids content of the extracts were determined by using aluminium chloride colorimetric method in reference with the modified procedure.[16,17] Quercetin was used to make the standard calibration curve. 10mg of quercetin was dissolved in 80% of ethanol and diluted to 10, 20, 40, 60, 80, 100µg/ml. The diluted standard solution (0.5ml) were mixed with 1.5ml of 95% ethanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415nm. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5ml of the test extracts were reacted with aluminium chloride for determination of flavonoids content as described above. The total flavonoids contents were expressed as milligrams of quercetin equivalents per gram extract.

## *In vitro* antioxidant activity

*In vitro* antioxidant evaluation of the leaves and stem extracts were done by different approaches such as DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, reducing power assay, superoxide and nitric oxide radical scavenging assays.

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

DPPH assay of the tests was carried out with reference.[13] Reaction mixture contains 1ml of test extracts (800µg/ml) and 1ml of methanolic solution of DPPH (0.3mM). It was kept in 30min. incubation in dark at room temperature. Measuring of absorbance was done at 517nm, using methanol as the blank, DPPH in methanol as the control and ascorbic acid (800µg/ml) as the standard control. The inhibition percentage of DPPH radical by the tests was identified by

$$\% \text{ inhibition} = \frac{\text{Abs. (Control)} - \text{Abs. (Sample)}}{\text{Abs. (Control)}} \times 100$$

## Reducing power assay

Evaluation by reducing power assay method was done in reference with the modified procedure.[13,18-20] 1ml of the test extracts (800µg/ml) were mixed with sodium phosphate buffer (2.5ml; 0.2M; pH 6.6) and potassium ferricyanide (2.5ml; 1%) and incubated (50°C; 20min). Then, trichloro acetic acid (2.5ml; 10%) was added to the incubated mixture and centrifuged (3000rpm; 10min; 4°C) and the supernatant was collected. 2.5ml of the supernatant solution was mixed with equal volume of distilled water and fresh ferric chloride solution (0.5ml; 0.1%) and kept for 10min. Measuring the absorbance of reaction mixture was done at 700nm. Ascorbic acid (800µg/ml) was used as the standard for comparative evaluation.

## Superoxide radical scavenging assay

The superoxide radical scavenging activity was assessed by using the modified procedure.[21-24] The reaction mixture contains 3ml of test extracts (800µg/ml), 0.1ml of 1.5mM Nitro blue tetrazolium solution, 0.2ml of 0.1M EDTA, 0.05ml of 0.12mM riboflavin and 2.5ml of 0.067M phosphate buffer. Control tubes were prepared with DMSO. The reaction mixture was kept in front of 34W fluorescent light for 30 min., then the absorbance was measured at 560nm. Ascorbic acid (800µg/ml) was employed as standard control. The percentage inhibition of superoxide radical was calculated by

$$\% \text{ inhibition} = \frac{\text{Abs. (Control)} - \text{Standard}}{\text{Abs. (Control)}} \times 100$$

## Nitric oxide radical scavenging assay

Evaluation was done by using modified procedure.[23,24] Griess reagent (1ml) was added to the reaction mixture (5ml) prepared by mixing 1ml of the test extracts (800µg/ml) with 5mM sodium nitroprusside in phosphate buffer (pH 7.3). This reaction mixture was kept in 25°C in front of 25W tungsten lamp for 3h. The nitric oxide radical thus formed was interacted with oxygen to produce nitrite ion which was measured spectrophotometrically at 540nm. Normal and standard control (Ascorbic acid) were prepared. The percentage inhibition of nitric oxide radical formation was determined by

$$\% \text{ inhibition} = \frac{\text{Abs. (Control)} - \text{Abs. (Sample)}}{\text{Abs. (Control)}} \times 100$$

# RESULTS

In the present study, the leaf and stem parts of *Aerva javanica* was shade dried, powdered separately in to coarse powder and extracted with ethanol. The ethanol extract of *A. javanica* leaves yielded 10.5gm and the stem material gave 9.1gm of the dried extract.

In the preliminary phytochemical analysis, both the leaves and stem extract showed the presence of alkaloids, glycosides, tannins and phenolic compounds and flavonoids. The results showed the absence of carbohydrates, proteins and amino acids and sterols (Table 1). The results of total phenolic estimation is shown in Table 2 and the Figure 1. The total phenolic content in the ethanol extract of *A. javanica* leaves and stems were found to be 891mg/g and 841mg/g of extract equivalent to gallic acid. The results of total flavonoids estimation is shown in Table 3 and the Figure 2. The total flavonoids content in the ethanol extract of

**Table 1: Preliminary phytochemical analysis of ethanolic extract of *A. javanica* leaves and stem.**

| Sl. No. | Chemical Test | EEAJL | EEAJS |
|---|---|---|---|
| **1** | **Alkaloids** | | |
| a | Mayer's test | + | + |
| b | Wagner's test | + | + |
| c | Hager's test | + | + |
| d | Dragendorff's test | + | + |
| **2** | **Glycosides** | | |
| a | Legal test | + | + |
| b | Baljet test | + | + |
| **3** | **Phenolic compounds and Tannins** | | |
| a | Ferric chloride test | + | + |
| b | Lead acetate test | + | + |
| **4** | **Flavanones and flavonoids** | | |
| a | Aqueous NaoH test | + | + |
| b | Ammonia test | + | + |
| c | Shinoda test | + | + |
| **5** | **Terpenoids** | | |
| a | Salkowski test | – | – |
| **6** | **Carbohydrates** | | |
| a | Molisch's test | – | – |
| b | Fehling's test | – | – |
| c | Benedict's test | – | – |
| **7** | **Proteins and Amino acids** | | |
| a | Millon's test | – | – |
| b | Biuret test | – | – |
| c | Ninhydrin test | – | – |
| **8** | **Sterols** | | |
| a | Libermann-Burchard test | – | – |
| b | Salkowski test | – | – |

(+) – Presence of active constituents; (–) – Absence of active constituents.

**Table 2: Estimation of total phenolic content in ethanolic extract of *A. javanica* leaves and stem.**

| Sl. No | Sample | Concentration (µg/ml) | Absorbance (750nm) |
|---|---|---|---|
| 1 | Standard (Gallic acid) | 10 | 0.027 |
| | | 20 | 0.069 |
| | | 40 | 0.128 |
| | | 60 | 0.179 |
| | | 80 | 0.251 |
| 2 | EEAJL | 40 | 0.109 |
| 3 | EEAJS | 40 | 0.115 |

*A. javanica* leaves and stems were found to be 950mg/g and 900mg/g of extract equivalent to quercetin.

Antioxidant activity of the leaves and stem extracts were evaluated by DPPH assay, reducing power assay, superoxide and nitric oxide radical scavenging assay methods. The leaves extract showed maximum antioxidant activity comparing with stem extract. The activity showed by the leaves extract is comparable with standard drug (Table 4).

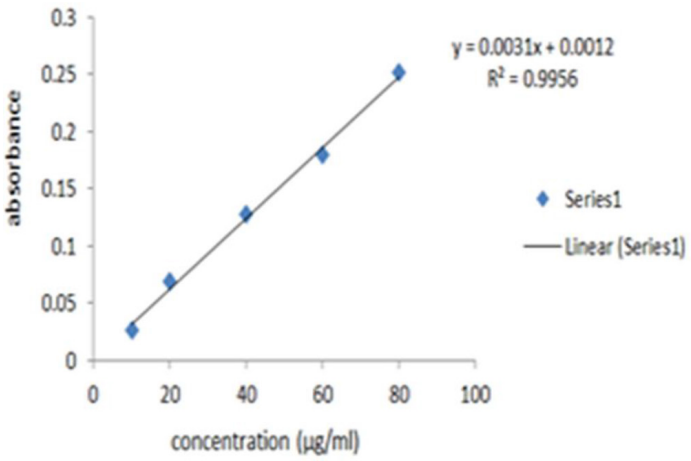


**Figure 1:** Calibration curve of gallic acid in the estimation of total phenolic content.

**Table 3: Estimation of total flavonoids content in ethanolic extract of *A. javanica* leaves and stem.**

| Sl. No | Sample | Concentration (µg/ml) | Absorbance (415nm) |
|---|---|---|---|
| 1 | Standard (Quercetin) | 10 | 0.018 |
| | | 20 | 0.150 |
| | | 40 | 0.496 |
| | | 60 | 0.661 |
| | | 80 | 0.963 |
| | | 100 | 1.181 |
| 2 | EEAJL | 20 | 0.125 |
| 3 | EEAJS | 20 | 0.113 |

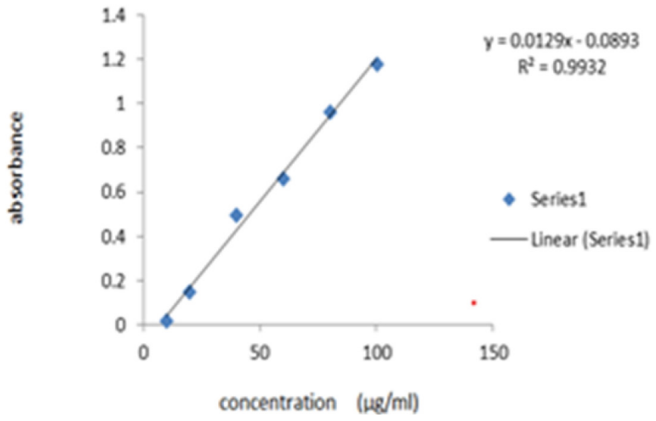


**Figure 2:** Calibration curve of Quercetin in the estimation of total flavonoids content.

## DISCUSSION

In the preliminary phytochemical evaluation, the presence of flavanones and flavonoids are identified in the extracts. This phytochemical is well

**Table 4: Antioxidant activity of ethanolic extract of *A. javanica* stem and leaves.**

| Tests | % inhibition | | |
|---|---|---|---|
| | EEAJS | EEAJL | Standard |
| DPPH assay | 67.21 ± 1.15 | 71.45 ± 3.01 | 84.15±2.21 |
| Reducing power assay | 59.31 ± 0.1 | 65.93 ± 0.20 | 79.24±3.25 |
| Superoxide radical scavenging assay | 60.18 ± 1.8 | 64.23 ± 1.65 | 81.30±2.10 |
| Nitric oxide radical scavenging assay | 63.10 ± 0.15 | 69.48 ± 0.12 | 75.43±3.15 |

known for its antioxidant activity[16] which is strongly documented in the results of present study. Regarding with antioxidant activity, the imbalance between cellular production of reactive oxygen species (ROS) and cellular capacity to scavenge them is known as oxidative stress (OS). OS has been mentioned as a possible factor in the aetiology of numerous disorders include diabetes, heart disease, and cancer.[6] Numerous cellular constituents, such as lipids, proteins, and nucleic acids like DNA, are damaged by ROS, which results in cellular death through necrosis or apoptosis. Due to compromised cellular antioxidant defence mechanisms, the damage may spread further.[7] Even low levels of oxidative stress are highly sensed and activate the protective antioxidant mechanism, which is crucial for maintaining the structural integrity of proteins. Antioxidants arrange many biologic responses like immunity. They also serve as signalling mechanisms for redox regulation.[25,26] Polyphenolic compounds in plants are responsible for very good antioxidant property. They are able to adsorb, neutralise, and quench free radicals, which is what causes this activity.[16] The presence phenolic compounds is reported in the results of preliminary phytochemical evaluation of the present study. The presence of alkaloids and glycosides also identified in the extracts. Alkaloids are well known for their anticancer activity by interfering with cell division. The presence of polyphenolic glycoside (flavonoids) was identified in the preliminary phytochemical evaluation of the present study.

## CONCLUSION

In the present study, the phytochemical and antioxidant property of the ethanolic extract of leaves and stem of *Aerva javanica* was evaluated successfully. A significant antioxidant activity of leaves extract was found in the study. The presence of flavonoids and phenolic compounds identified in the preliminary phytochemical evaluation is well known for their antioxidant activity which is confirmed by the results of present study. The presence of alkaloids and glycosides also identified in the extracts. Anticancer activity of alkaloids are well documented one. Hopefully, further studies in this direction may give more promising data. Moreover, the chromatographic and spectral studies of the extracts will reveal the details of active constituents present.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**DPPH – 2:** 2-diphenyl-1-picrylhydrazyl; **BHA:** Butylated hydroxyl anisole; **BHT:** Butylated hydroxyl toluene; **EDTA:** Ethylene diamine tetra acetic acid; **DMSO:** Dimethyl sulfoxide; **ROS:** Reactive oxygen species; **OS:** Oxidative stress.

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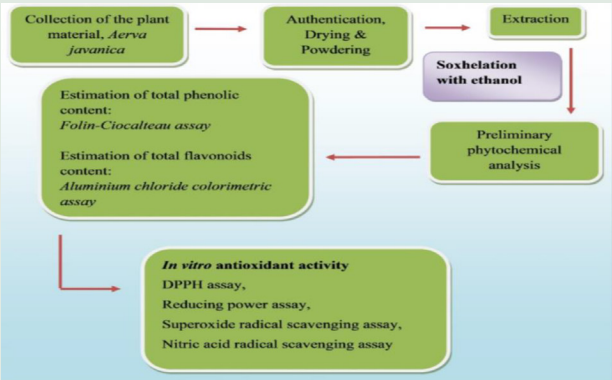
## GRAPHICAL ABSTRACT




## SUMMARY

- Collection, identification and authentication of *Aerva javanica* plant.
- Drying, powdering and ethanol extraction of leaves and stem of *A. javanica* plant.
- Preliminary phytochemical evaluation, Estimation of total phenolic (Folin-Ciocalteau assay) and flavonoids content (Aluminium chloride colorimetric assay) of the extracts.
- Evaluation of *in vitro* antioxidant activity of the extracts - DPPH assay, reducing power assay, superoxide radical scavenging assay, nitric acid radical scavenging assay.
- Leaves and stem extract of *A. javanica* showed the presence of alkaloids, glycosides, tannins and phenolic compounds and flavonoids. The results showed the absence of carbohydrates, proteins and amino acids and sterols.
- The total phenolic content in the ethanol extract of *A. javanica* leaves and stems were found to be 891mg/g and 841mg/g of extract equivalent to gallic acid.
- The total flavonoids content in the ethanol extract of *A. javanica* leaves and stems were found to be 950mg/g and 900mg/g of extract equivalent to quercetin.
- The leaves extract showed maximum antioxidant activity comparing with stem extract in all the test approaches employed.

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