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# PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF STEM EXTRACT OF*ANDROGRAPHIS PANICULATA* (BURM.F.) NEES

**D. Jayapriya[1], L. Jeyanthi Rebecca[2*], P. Arumugam[3] and C. Sivaraj[4]**
[1]Research Scholar, Department of Biotechnology and Microbiology, BIHER, Chennai
[2]Professor, Department of Industrial Biotechnology, BIHER, Chennai
[3]Director ArmatsBiotek, Chennai
[4]Research Analyst, ArmatsBiotek, Chennai

## ABSTRACT

Medicinal plants have been used as a source of medicine to alleviate diseases. *Andrographis paniculata* is one of the highly used potential medicinal plants in the world. This plant is traditionally used for the treatment of common cold, diarrhoea, fever due to several infective cause, jaundice, as a health tonic for the liver and cardiovascular health, and as an antioxidant. It is also used as a contraceptive. All parts of this plant are used to extract the active phytochemicals, but the compositions of phytoconstituents widely differ from one part to another and with place, season, and time of harvest. The aim of the study was to evaluate the Phytochemical analysis, antioxidant, antibacterial activities and GCMS analysis of stem extract of *Andrographis paniculata*. The Phytochemical analysis of *A. paniculata* reveals the presence of alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, glycosides, tannins and saponins. Antioxidant assays such as DPPH$^{\bullet}$ radical, superoxide radical, phosphomolybdenum reduction and $Fe^{3+}$ reducing power assays were carried out for evaluating antioxidant activities. The maximum DPPH• radical scavenging activity of *A. paniculata* stem extract was 66.99±0.75% at 300 µg/mL concentration. The maximum superoxide radical scavenging activity of stem extract was 77.90±0.34% at 120 µg/mL concentration. The maximum $Mo^{6+}$ (molybdenum) reduction of stem extract was 79.86±0.87% at 300 µg/mL concentration. The maximum $Fe^{3+}$ reduction was 45.17±1.37% at 120 µg/mL concentration. The maximum α-amylase enzyme inhibition of stem extract of *A. paniculata* was 57.70 ±0.97% at 120 µg/mL concentration. The maximum hemolysis inhibition was 54.00±1.16 at 120 µg/mL concentration. Antibacterial activity was carried out by well diffusion method and the stem extract showed

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maximum zone of inhibition of 20 mm against *Staphylococcus aureus* at 500 µg/mL concentration.

***Keywords:*** Phytochemicals, Antioxidant, Antibacterial, DPPH radical.

**Introduction**

Medicinal plant is an integral part of human life to combat the sufferings from the dawn of civilization. The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various health ailments. Use of the medicinal plants is a core component at primary health care level due to availability, acceptability, compatibility, and affordability. Dependency on these medicinal plants varies from country to country. It is estimated that about 75–80% of people of developing countries and about 25% of people of developed countries depend either directly or indirectly on medicinal plants for the first line of treatment .Therefore people are encouraging indigenous production and processing of these medicinal plants to use in different cultures and religion for the treatment of various diseases This plant has been mentioned in Charaka Samhita, Holy Quran and Holy Bible for its immense medicinal properties. *A. paniculata*, also known as Kalmegh, Bhunimba (Sanskrit) or Nilavembu (Tamil/Telugu) or Kirayat/Kalpnath (Hindi) or king of bitters (English)*Andrographis paniculata*, commonly known as creat or green chiretta, is an annual herbaceous plant in the family Acanthaceae, native to India and Sri Lanka.

*A.paniculata* (Fig 1) is an important medicinal plant and widely used around the world. *A.paniculata* is used as a traditional herbal medicine and is ethnobotanically used for the treatment of snake bite, bug bite, diabetes, dysentery, fever, and malaria .In recent times, commercial preparations of this plant extracts are also used in certain countries. However, the preparations yet need to be standardized for their better efficacy. The aerial part of *A.paniculata* is most commonly used and its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides. Whole plant leaves and roots are also used in remedy for different diseases. *A.paniculata* has been reported to have a broad range of pharmacological effects including anticancer, antidiarrheal, antihepatitic, anti-HIV, antihyperglycemic,anti-inflammatory, antimicrobial, antimalarial, cardiovascular cytotoxic, hepato protective, immunostimulatory and some other dysfunctions. It is used for the treatment of diseases including diarrhea, upper respiratory infection (common cold, flu), liver disease (enlargement of

  

liver, jaundice), fever, cardiovascular disease, dyspepsia, skin infection, colic dysentery. It is also consumed as health tonic because of its antioxidant property.

The Phytochemical analysis of *A. paniculata* reveals the presence of alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, glycosides, tannins and saponins. Antioxidant assays such as DPPH$^{\bullet}$ radical, superoxide radical, phosphomolybdenum reduction and $Fe^{3+}$ reducing power assays were carried out for evaluating antioxidant activities. A broad range of natural products is used in cosmetics preparations, skin care such as treatment of dryness, treatment of eczema and acne, as well as antioxidant, anti-inflammatory, anti-aging, hair care products such as hair growth imputes, hair color, scalp complaints like dandruff, and skin protection and also toiletry preparations. *A.paniculata* (Burm.f.) has long been used in ayurvedic medicine through its inflammatory properties.

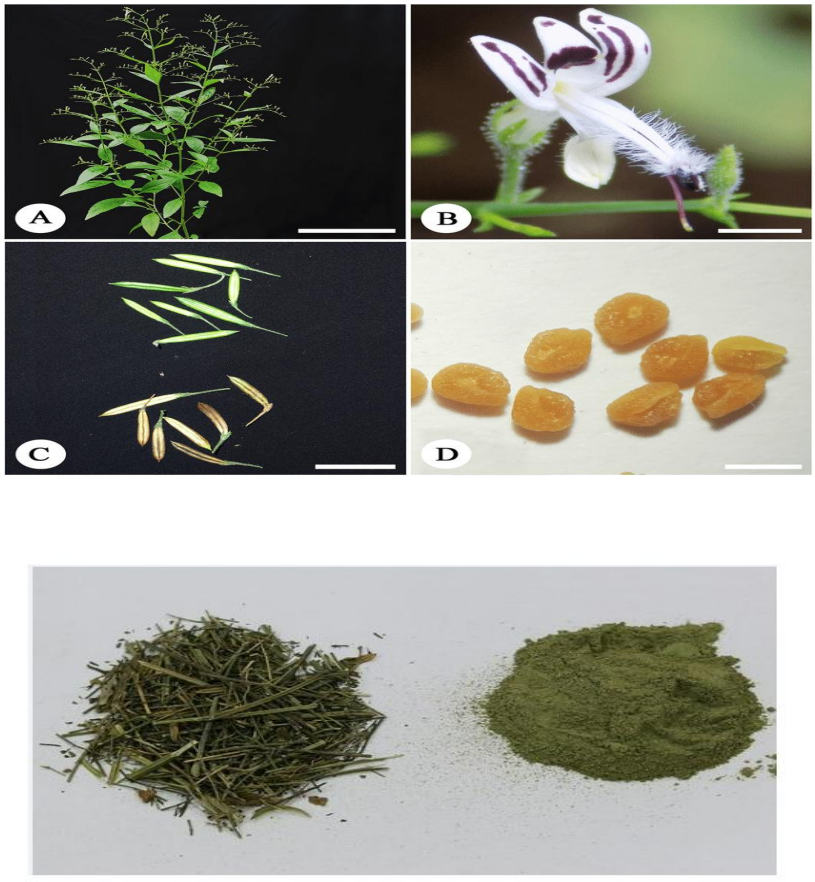


**Fig 1** Flower, leaf, seeds, dried and powdered stem of *Andrographis paniculata*

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## MATERIALS AND METHODS

### Preparation of Extract

*A.paniculata* plants were collected at Singaperumal Koil, Chengalpattu district, Tamilnadu, India. The stems were removed and shade dried for 5 days. Then, the stem parts were powdered and about 20 g of *A.paniculata* powder was soaked in 200 mL of methanol in a conical flask for 72 h. The supernatant was filtered and condensed at 50°C, which yields greenish gummy extract.

### Qualitative phytochemical screening

The different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition (Trease and Evans 1983). The tests were done individually for crude methanol extract.The following tests were performed on the extracts to detect various phytoconstituents present in them (Raaman, 2006).

### Detection of alkaloids (Evans, 1997)

Solvent free extract (50 mg) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows:

### Mayer's test (Evans, 1997)

To a few mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

### Dragendorff's test (Waldi, 1965)

To a few mL of filtrate 1 or 2 ml of Dragendorff's reagent was added. A prominent orange precipitate indicates the test positive.

### Detection of Terpenoids

To 5 mL of methanolic extract, 2 mL of chloroform was added and mixed well. Add a little of conc.$H_2SO_4$ was carefully added to form a reddish brown layer

### Detection of phytosterols (Finar, 1986)

### Libermann-Burchard's test

The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated $H_2SO_4$were added slowly along the sides of test tube. An array of colour changes showed the presence of phytosterols.

### Detection of phenolic compounds

### Ferric chloride test (Mace, 1963)

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

**Detection of Flavonoids**

To the methanolic extract, $NH_3$ solution or NaOH solution was added. Bright yellow color appears and addition of excess amount of con.$H_2SO_4$, the yellow color disappears.

**Detection of Tannins**

**Lead acetate test**

The extract (50 mg) was dissolved in distilled water and to this; 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

**Detection of glycosides**

**Legal's test**

Alcoholic extract was dissolved in 2 ml of pyridine and sodium nitroprusside 2 ml was added followed by addition of NaOH solution to make alkaline. Formation of pink colourindicated the presence of glycosides.

**Detection of carbohydrates**

**Molisch test**

To the extract, two drops of alcoholic α-naphthol solution was added and shaken well. To this, few drops of Conc.$H_2SO_4$ was added. Formation of violet ring indicated the presence of carbohydrates.

**Detection of saponins**

**Foam test (Kokate, 1999)** The extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

**Phytochemical Estimation**

**Determination of total phenols**

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds with slight modifications (Spanos and Wrosltad 1990). One hundred µL methanol stem extract of *A.paniculata* (1mg/mL) was mixed with 900 µL of distilled water and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of 20% (w/v) of $Na_2CO_3$ solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room

  

temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

**Determination of total flavonoids**

The total flavonoid content of methanol stem extract of *A.paniculata* was determined using aluminum chloride method with slight modification as described by Liu et al., 2007. Five hundred µL of extract (1mg/mL) was mixed with 0.5 mL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution. Then, 0.5 mL of 10% (w/v) aluminum chloride solution was added, shaken well, followed by 100 µL of 1 M NaOH solution was added. The absorbance was measured at 510 nm and the result was expressed as (µg/mg of extract) quercetin equivalent, which is a common reference compound.

***In vitro* antioxidant assays**

**DPPH˙ radical scavenging activity**

The antioxidant activity of methanol stem extract of *A.paniculata* was measured by DPPH (1, 1-diphenyl 2-picrylhydrazyl) free radical scavenging activity (Blois, 1958). One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of methanol stem extract. The mixture was then allowed to stand for 30 min incubation in dark. One mL of DPPH solution mixed with 1 mL of methanol was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

**Superoxide radical scavenging activity**

Superoxide radical scavenging activity was carried out by the method of Ravishankara*et al*., 2002. The reaction mixture contains different concentrations (20-120 µg/mL) of methanol stem extract of *A.paniculata*,1.5 mM of riboflavin (200 µL), 12 mM of EDTA (100 µL), and 50 mM of NBT (50 µL). All the reagents should be prepared in 50 mM of phosphate buffer (pH 7.8). The reaction was started by illuminating the reaction mixture for 90 seconds. Immediately, the absorbance was measured at 590 nm and ascorbic acid was used as the positive control

$$\% \text{ of superoxide radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

**Phospho molybdenum reduction assay**

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The antioxidant capacity of methanol stem extract of *A.paniculata* was assessed as described by Prieto *et al*., 1999. Extract with different concentrations (20 - 120 µg/mL) was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 95˚C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of phosphomolybdenum reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

**Ferric ($Fe^{3+}$) reducing power assay**

The reducing power of methanol stem extract of *A.paniculata* was determined by the slightly modified method of Yen and Chen, 1995. One mL of extract with different concentrations (20 - 120 µg/mL) was mixed with 1 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixtures were then incubated at 50°C in a water bath for 20 min. Five hundred µL of 10% (w/v) trichloroacetic acid solution was added to each mixture followed by 1 mL of 0.1% (w/v) freshly prepared $FeCl_3$ solution was added and shaken well. The absorbance was measured at 700 nm and ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

**Antidiabetic activity**

**Alpha amylase enzyme inhibition assay**

α- amylase enzyme inhibition assay was carried out based on the starch-iodine test (Xiao *et al*., 2006). The total assay mixture was composed of various concentration (20-120 µg/mL) of methanol stem extract of *A.paniculata*, 10 µLof alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and was incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reactionset and incubated at 37°C for 60 min. One hundred µL of 1 M HCl was added to stop the enzymatic reactionand followed by 200 µL of iodine reagent (5 mM I 2 and 5 mM KI) was added. The colour change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did notcontain any plant extract. A dark-blue colour indicates the presence of starch; a yellow

  

colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor indicating that starch is completely hydrolyzed by α-amylase.

$$\% \text{ of } \alpha\text{- amylase enzyme inhibition} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

**Anti InflammatoryActivity**

**Membrane stabilization assay**

**Preparation of Red Blood cells (RBCs) suspension**

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 min and discard the pale yellow plasma. Then, about 10 mL of PBS was poured into centrifuge tube, centrifuged at 3000 rpm for 5 min, discard and discard the supernatant and repeat the washing process once again. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 40% v/v suspension with isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4). The buffer solution contained 0.2 g of $NaH_2PO_4$, 1.15 g of $Na_2HPO_4$ and 9 g of NaCl in 1 litre of distilled water. The reconstituted red blood cells (resuspended supernatant) were used as normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline.

**Heat induced haemolysis**

The reaction mixture (2 mL) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) and 1 mL of 10% RBCs suspension, instead of test sample only saline was added to the control test tube (Shinde *et al.*, 1989). All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. Aspirin was used as a standard drug.

The Percentage inhibition of Haemolysis was calculated as follows:

$$\text{Percentage of hemolysis inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

**Antibacterial activity**

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The microorganisms of Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis* andGram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were used for the evaluation of the antibacterial activity.

**Agar well diffusion method**

Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast extract-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g). The required amount of ingredients was weighed and suspended in the required volume of 100 mL of distilled water and then autoclaved at 15 lbs and 121ºC for 15 min. The hot medium was poured in sterile petri plates which were kept in the aseptic laminar chamber. The medium was allowed to solidify for 15 min.

Antibacterial activity of methanol stem extract of *A. paniculata*was carried out using agar well diffusion method (Kubo et al., 2002). The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which are previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The root tuber extract was then poured into each well containing 250, 375, and 500 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was used as the positive control.

**RESULTS AND DISCUSSION**

**Phytochemical analysis**

The Phytochemical analysis (Table 1, Fig 2) of *A. paniculata* reveals the presence of alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, glycosides, tannins and saponins which are responsible for antioxidant, antibacterial, antiinflammatory and antitumour properties (Chandra and Gonzalez de Mejia, 2004)

**Table 1:** Phytochemical analysis of methanol stem extract of *Andrographis paniculata*

| **Phytochemicals** | **Test** | **Inference** | **Result** |
|---|---|---|---|
| Alkaloids | Dragendorff's test | No yellow precipitate | + |
| Terpenoids | Salkowski test | Red ring appears. | + |
| Steroids | Libermann-Burchard's test | Dark violet colour appears | + |
| Flavonoids | Alkaline Reagent test | Yellow colour appears | + |
| Phenolic | $FeCl_3$ test | Violet or blue or green | + |

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| | | | |
|---|---|---|---|
| compounds | | colour appears | |
| Tannins | Lead acetate test S | White colour appears | + |
| Glycosides | Legal's test | Blood red color appears. | + |
| Carbohydrate | Molisch test | Violet ring appears | + |
| Saponins | Foam test | Foam appears | + |

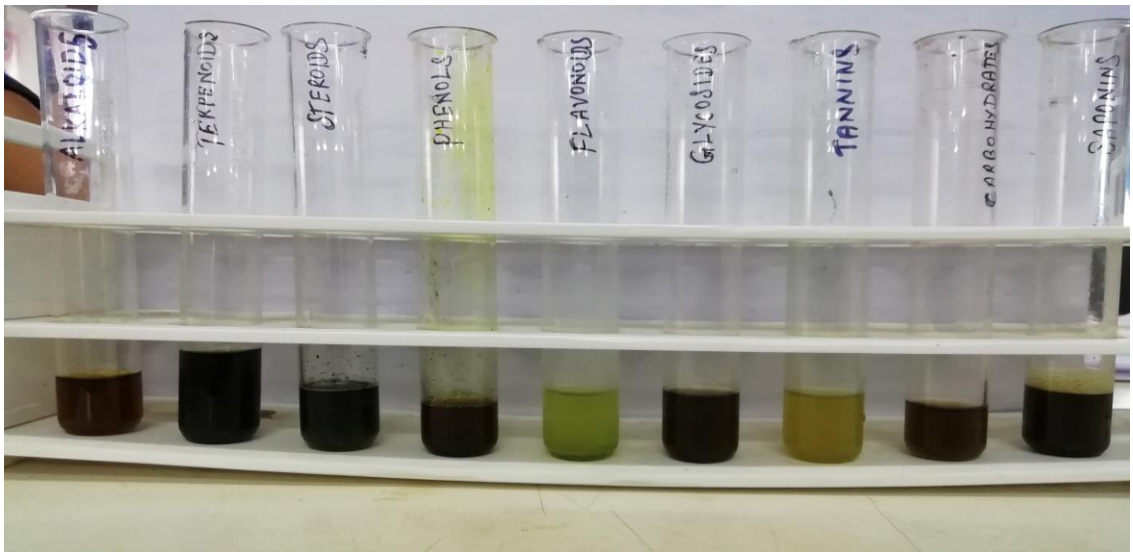


**Fig 2:** Phytochemical analysis of methanol stem extract of *Andrographis paniculata*

**Antibacterial activity by agar well diffusion method**

The methanol stem extract of *A.paniculata* was tested for its antibacterial activity against Gram-positive bacteria such as *S. aureus, E. faecalis* and Gram negative bacteria such as *E. coli* and *P. aeruginosa.*The antibacterial activity showed a maximum zone of inhibition of 20 mm against *Staphylococcus aureus* at 500 µg/mL concentration of the extract (Table 2, Fig 3). The antibacterial activity may be due to the presence of phenolic compounds, terpenoids, which inhibit bacterial cell wall biosynthesis by binding to a highly conserved motif of lipid II and lipid III, two key precursors of bacterial cell-wall polymers as peptidoglycan and teichoic acid that adversely affect the growth of microbes (Obeidat *et al*., 2012).

**Table 2**: Antibacterial activity of methanol stem extract of *Andrographis paniculata*

| **Organism** | **Zone of inhibition (mm)** | | | |
|---|---|---|---|---|
| | **250µg** | **375 µg** | **500µg** | **Standard (Tetracycline-40 µg)** |

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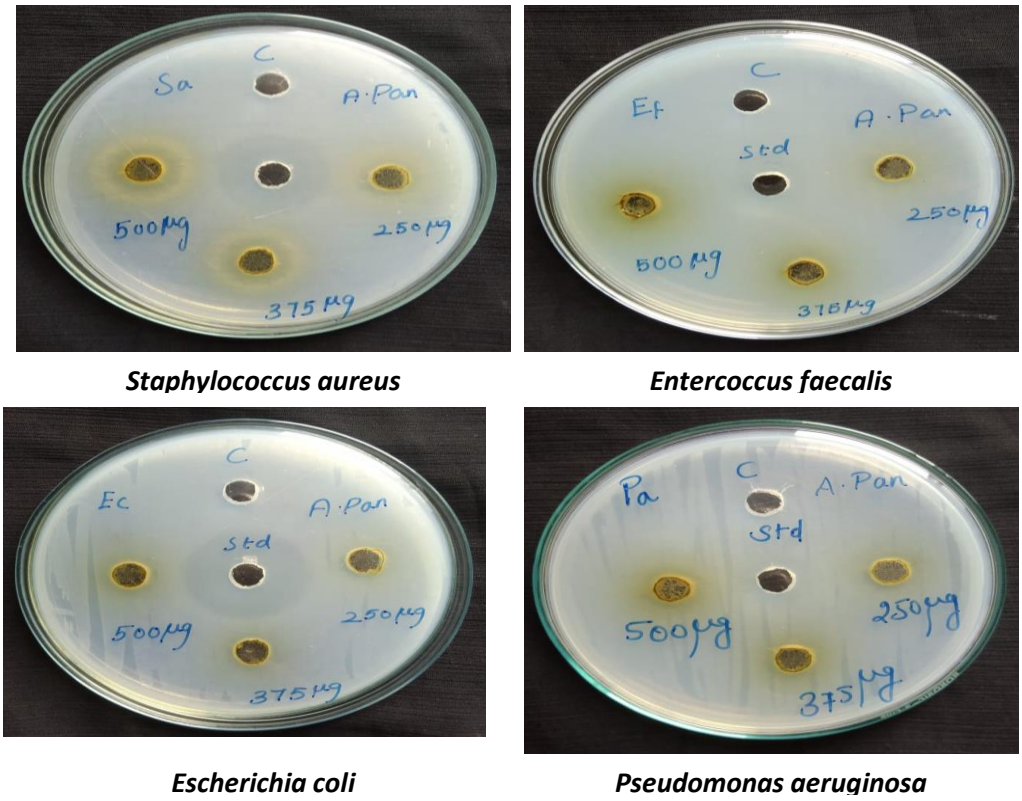
| | | | | |
|---|---|---|---|---|
| *Staphylococcus aureus* | 17 | 18 | 20 | 20 |
| *Entercoccus faecalis* | 14 | 17 | 19 | 17 |
| *Escherichia coli* | 10 | 11 | 12 | 22 |
| *Pseudomonas aeruginosa* | 13 | 15 | 16 | 14 |




***Staphylococcus aureus*** ***Entercoccus faecalis***

***Escherichia coli*** ***Pseudomonas aeruginosa***

**Fig 3:** Antibacterial activity of methanol stem extract of *Andrographis paniculata*.

It shows zone of inhibition around the disc at different concentration of methanol stem extract of A.paniculata.Zone of inhibition formed against *S. aureus, E. faecalis,E. coli, P. aeruginosa* (Fig 3).

**Table 3:** Quantitative estimations of methanol stem extract of *Andrographis paniculata*

| **S. No** | **Phytochemicals** | **Amount(µg/mg)** |
|---|---|---|
| 1. | Phenols | 198.39±0.14 |
| 2. | Flavonoids | 100.76±0.33 |

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**Table 4:** DPPH$^{\cdot}$ radical scavenging activities of methanol stem extract of *Andrographis panicula*

| | **Concentration ((µg/mL)** | **% of inhibition** |
|---|---|---|
| | | **DPPH$^{\cdot}$ radical** |
| 1 | 50 | 10.76 ±0.52 |
| 2 | 100 | 34.12 ±0.36 |
| 3 | 150 | 47.61±0.57 |
| 4 | 200 | 61.11±0.64 |
| 5 | 250 | 63.49±0.64 |
| 6 | 300 | 67.46±0.75 |

**Table 5**: Superoxide ($O_2^{\cdot -}$) radical scavenging activity of methanol stem extract of *Andrographi spaniculata*

| **S. No** | **Concentration (µg/mL)** | **% of inhibition** |
|---|---|---|
| | | **Superoxide ($O_2^{\cdot -}$) radical** |
| 1 | 20 | 31.08±0.52 |
| 2 | 40 | 43.82±0.59 |
| 3 | 60 | 52.43±0.53 |
| 4 | 80 | 67.41±0.58 |
| 5 | 100 | 76.40±0.54 |
| 6 | 120 | 77.90±0.34 |

**Table 6:** Phosphomolybdenum reduction activity of methanol stem extract of *Andrographis paniculata*

| **S.No** | **Concentration (µg/mL)** | **% of reduction** |
|---|---|---|
| | | **$Mo^{6+}$ reduction** |
| 1 | 50 | 48.96±1.30 |

 

| | | |
|---|---|---|
| 2 | 100 | 53.54±0.49 |
| 3 | 150 | 57.85±0.79 |
| 4 | 200 | 61.18±1.13 |
| 5 | 250 | 68.44±0.90 |
| 6 | 300 | 79.86±0.87 |

**Table 7:** Ferric ($Fe^{3+}$) reducing power activities of methanol stem extract of *Andrographis paniculata*

| **S. No** | **Concentration (µg/ mL)** | **% of reduction** |
|---|---|---|
| | | **$Fe^{3+}$ reduction** |
| 1 | 20 | 23.27±0.83 |
| 2 | 40 | 34.84±0.65 |
| 3 | 60 | 36.25±0.83 |
| 4 | 80 | 37.66±1.04 |
| 5 | 100 | 43.67±1.02 |
| 6 | 120 | 45.17±1.37 |

**Table 8:** Alpha amylase enzyme inhibition assayof methanol stem extract of *Andrographis paniculata*

| **S. No** | **Concentrations** | **% of inhibition** |
|---|---|---|
| | | **Alpha amylase enzyme** |
| 1 | 20 | 14.19±1.07 |
| 2 | 40 | 15.93±0.88 |
| 3 | 60 | 36.24±1.04 |
| 4 | 80 | 41.92±0.93 |
| 5 | 100 | 49.53±1.14 |
| 6 | 120 | 57.70±0.97 |

Tables 3 to 8 illustrate the phytochemical constituents and antioxidant activity of *A. paniculata*.

## CONCLUSION

People from worldwide have been using plant-based substances to enhance the appearance since the existence of mankind. In the ancient Egypt, around 3000 BC, there is evidence of using cosmetics, and their usages have been a necessary part in our everyday life in all

  

cultures. Initially, natural products have been used for beauty products; occasionally augment with paints and dyes. Natural products have approached back with present trend cosmetic products which are mainly derived from plant sources. Since from longer time, plant products (Natural Products) are source of food and medicines. A broad range of natural products is used in cosmetics preparations, skin care such as treatment of dryness, treatment of eczema and acne, as well as antioxidant, anti-inflammatory, anti-aging, hair care products such as hair growth imputes, hair color, scalp complaints like dandruff, and skin protection, and also toiletry preparations. Essential oils are major source of plants; essential oils have been used in preparation of perfumes, hair care substances, emollient of the skin. This study demonstrated that stem extract of *Andrographis paniculata* has significant antioxidative activity *in vitro*. The antiinflammatory activity by Human red blood cell membrane stabilization (HRBC method) assay showed good membrane stabilizing activity.

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