

# PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL, ANTIFUNGAL AND ANTIINFLAMMATORY ACTIVITY OF HUMIC ACID

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**ABSTRACT:** Humic acid is a prolific constituent of humic substances and it is distributed throughout the environment. This humic acid is known as black gold of agriculture. Humic acid contributes to the growth of plants and is responsible for the structure and physio-chemical properties of soil. Humic substances can be fractionated into fulvic acids, brown humic acids, and grey humic acid in humin as a function of their solubility at different pH values. Humic and fulvic acid contains large amount of a wide variety of trace minerals. Several humic substances have been identified based on their solubility, molecular size and composition. It also promotes the growth of plants. This humic substance has many medicinal properties and is used as a traditional remedy for a variety of illness in many countries. Hence, this study was carried out on phytochemical analysis of humic acid and to find the effect of anti-inflammatory, anti-microbial and anti-fungal activity of humic acid.

**Key Words:** Humic acid, phytochemical analysis, anti-bacterial, anti-fungal, anti-inflammatory activity.

## INTRODUCTION

Humic acids are component of humic substances which are the major organic constituents of soil (humus), peat and coal. It is a major organic compound of many upland streams, dystrophic lakes and oceans [Stevenson F.J, 1994]. Humic acids are formed by the microbial degradation of dead plant matter such as lignin and charcoal [Ponomarenko *et al.*, 2001 & Mao *et al.*, 2012]. Humic acids can form complexes with ions that are commonly found in the environment creating humic colloids. Humic acids are insoluble in water at acidic pH, whereas fulvic acids derived from humic substances, are soluble in water across the full range of pH [MacCarthy *et al.*, 2001]. There is a growing interest in the use of humic acid as organic fertilizers or soil tonic [Khalid Mehood *et al.*, 2012]. Soil microbes are responsible for solubilizing vital nutrients such as phosphorus which can be absorbed by the humic acid and in turn made available to the plant [Peregrine Phosphate Private Limited]. Thus, it promotes plant growth and soil quality. Humates are the raw material that can be used in agriculture and animal husbandry in the form of a humate drink or as dry feed, a source of mineral and organic substances of growth stimulation [Achard *et al.*, 1986]. Humic and fulvic acids are commonly used as soil in agriculture and less commonly as a human nutritional supplement [Yamauchi *et al.*, 1984]. Humic acid possess medicinal properties which is useful for mankind for treatment of several ailments.

## MATERIALS AND METHODS

### Collection of sample

It is a readily available sample which is obtained from the chemical laboratory.

### Estimation of phenol (Folin's ciocalteau method)

To 0.2 ml of test sample, added 1ml Folin's reagent and 0.8ml of 7.5% Sodium bicarbonate after 10 minutes placed it in dark for 30 minutes. This reaction turns the sample into blue in color. The color intensity is proportional to the concentration of phenol. At the end of incubation, the readings are noted for blank and sample solution at 743nm in colorimeter.

### ANTI-MICROBIAL ACTIVITY

The Humic acid were individually tested against a set of fungal and bacterial strains; *Aspergillus niger*, *Aspergillus fusarium*, *Aspergillus terreus*, *Aspergillus flavus*, *Staphylococcus Aureus*, *Pseudomonas Aeruginosa*, *Klebsiella Pneumonia*, *Proteus Vulgaris*. Nutrient agar was used for bacterial and fungal growth, respectively. The antimicrobial activity of humic acid was determined using agar well diffusion method [Chung *et al.*, 1990 & Bauer *et al.*, 1996]. Nutrient agar was suspended in 200ml of distilled water, mixed well and distributed homogeneously. The medium was sterilized by autoclaving at 121°C for 15 min. Medium was transferred into sterilized petri plates. After that, 10µl of inoculum was added to the medium. Two wells

were created and 1ml of microbial cultures was inoculated and samples were added (Sample I: 3g, Sample II: 6g).The Petri plates were then incubated (37°C for bacteria and 30°C for fungus) for 24 hours. The humic acid having antimicrobial activity inhibited the bacterial and fungal growth, and clear zones were formed after 24 hours which were measured in mm using zone reader.

### **ANTI-INFLAMMATORY ACTIVITY BY HRBC SUSPENSION METHOD**

#### **Chemicals**

Isosaline (0.85 w/v Sodium chloride), Hyposaline (0.75 % Sodium chloride) , Phosphate buffer pH 7.4, Alsever solution: (2% dextrose,0.8% sodium citrate,0.05% citric acid,0.42% sodium chloride).

#### **Procedure**

Blood was collected from healthy human volunteers who had not consumed any non-steroidal anti-inflammatory drugs for 2 weeks prior to the experiment. Blood was mixed with equal volume of Alsever solution and centrifuged with isosaline at 3000 rpm for 10 minutes at room temperature. The supernatant was carefully removed while the packed red blood cell was washed in fresh normal saline (0.85% w/v Sodium chloride) and centrifuged till the supernatant was clear.Pellet was taken and prepared 10% suspension (1ml of pellet and 9ml of isosaline).Added 2ml of Hyposaline and 1ml of phosphate buffer and 0.5 ml of suspension and 100µl of humic acid mixture was taken in the Eppendorf tube. Kept in room temperature for 5 minutes and taken reading was recorded at 560nm.

### **PHYTOCHEMICAL ANALYSIS**

#### **Test for Flavonoid**

1ml of humic acid sample was treated with few drops of 1% Sodium hydroxide, the change in color was observed. After a minute few drops of dilute Hydrochloric acid was added. The disappearance of the color was observed. The appearance of yellow color after adding Sodium hydroxide indicates presence of flavonoids.

#### **Test for Quinone**

To 1ml of humic acid sample in a test tube added few drops of concentrated hydrochloric acid. The appearance of yellow precipitate indicates the presence of Quinone.

#### **Test for Tannins**

To 1ml of humic acid sample in a test tube added 10ml of distilled water. This sample was filtered and added few drops of 0.1 % ferric chloride, then the change in color was observed. The appearance of brownish color or blue/black color indicates the presence of Tannins.

#### **Ferric chloride test for Phenol**

To 1ml of humic acid sample in a test tube added 20µl of 1% Ferric chloride solution. The appearance of blue color or bluish black precipitate indicates the presence of phenols.

### **RESULTS AND DISCUSSION**

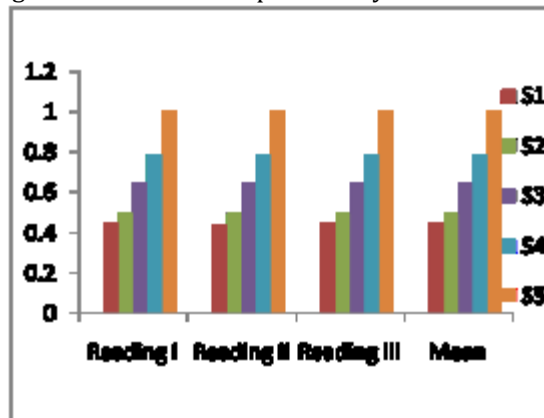
#### **Estimation of phenol's by Folin's Ciocalteu**

The result of estimation of phenol by Folin's ciocalteu clearly showed the presence of phenol.Phosphomolybdic acid and phosphotungstic acids in the Folin's phenol reagent is oxidized to produce blue color.The present study revealed the presence of Flavonoids in humic acid which shows with the gradual increase and higher amount of flavonoids.Polyphenols in plant extracts react with specific redox reagents (Folin's-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [Schofield P *et al.*, 2001]. The reaction forms a blue chromophore constituted by a phosphotungstic phosphomolybdenum complex [Gulçin I *et al.*, 2004], where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds [Schofield P *et al.*, 2001].

Del-Rio *et al* found that flavonoids are potential water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [Del-Rio *et al.*, 1997].

Duraipandiyar *et al* also found flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergies, anti-inflammatory, anti-microbial and antidiabetic activities [Duraipandiyar V *et al.*, 2006].

Figure: 1 Estimation of phenol's by folin's ciocalteu



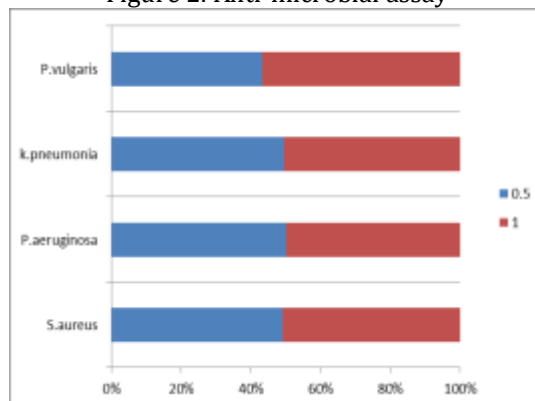
**ANTI-MICROBIAL ASSAY**

Anti-microbial assay in humic acids showed the positive results on these *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris* organisms.

Table 1: Anti-microbial assay on humic acids

ORGANISM	0.5 (WELL PUNCTURE)	1.0 (WELL PUNCTURE)
<i>Staphylococcus aureus</i>	1.13mm	1.17mm
<i>Pseudomonas aeruginosa</i>	1.19mm	1.18mm
<i>Klebsiella pneumonia</i>	2.11mm	2.14mm
<i>Proteus vulgaris</i>	2.15mm	2.12mm

Figure 2: Anti-microbial assay



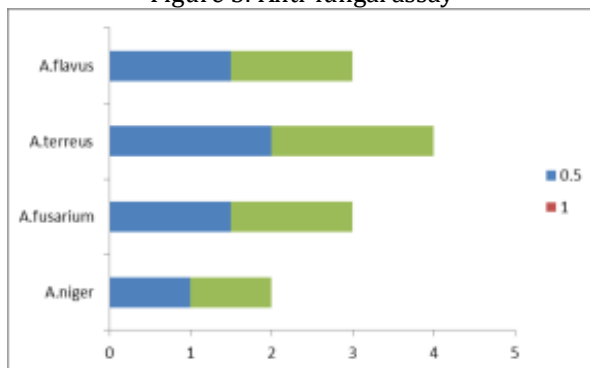
The properties of humic substances represent new possibilities for their medical application [Ansorg *et al.*, 1978 & Skliar *et al.*, 1998]. Humates are known to stimulate microbial activity. In soil testing for microbial activity, the levels increased 400 to 5000 times with the introduction of humate (300 ppm) into the soil. Humates added to feed rations stimulate the microbial growth and the extent can be quite large depending upon the species, the culture medium, and the environment [Huck *et al.*, 1991].

Table 2: Anti-fungal assay on humic acids

ORGANISM	0.5 (WELL PUNCTURE)	1.0 (WELL PUNCTURE)
<i>Aspergillus Niger</i>	1.0mm	1.0mm
<i>Aspergillus fusarium</i>	1.5mm	1.5mm
<i>Aspergillus terreus</i>	2.0mm	2.0mm
<i>Aspergillus flavus</i>	1.5mm	1.5mm

The research indicated the antifungal activity of positive correlation with the inhibition rates of HAS against fungi.

Figure 3: Anti-fungal assay



Organisms were taken place in the activity given the zone of equivalence level can be measured. *Aspergillus terreus* is the highest yield in this activity.

**ANTI-INFLAMMATORY ACTIVITY**

The anti-inflammatory activity gave the clear-cut transmission of the suspension.

Figure 4: Anti-inflammatory activity



It shows the reaction of living tissues towards injury. Humic substances were found to exhibit many biological activities such as anti-inflammatory and pro-inflammatory properties [June R. *et al.*, 2009]. Humic acids isolated from peat exhibited significant efficacy for adhesions when tested on female rats that had standardized lesions placed on both uterine horns and the peritoneum of the anterior abdominal wall [Yang *et al.*, 1996]. Humic substances, including peat and sodium humates, are known to exhibit anti-inflammatory properties [Kuhnert M. *et al.*, 1982]. Inflammatory states of the cervix, especially cervical erosion (generally known as cervicitis) can be treated with humic preparations [Jankowski A *et al.*, 1993]. The humate relieves swelling from joint inflammation and it has been shown to bond to the collagen fibers to aid in repair of damaged tendons and bone. Tendon strength has been shown to increase by as much as 75% [Iubitskaia *et al.*, 1999 & Kreutz *et al.*, 1992]. Humic acids have also been successfully used as anti-inflammatory agents because of their local anti-inflammatory, hyperaemic and analgesic properties [Salz, 1974; Motohisa *et al.*, 1974] and they are also used as a systemic treatment for anaemia and hypercholesterolemia [Soloveyva and Lotosh.,1984].

**PHYTOCHEMICAL ANALYSIS**

The results of phytochemical analysis of humic acid clearly showed the presence of Flavonoids, Quinone, Tannins and phenol.

Table 3: Phytochemical Screening

S.No	Phytoconstituents	Results
1	Flavonoids	+++
2	Quinone	+++
3	Tannins	+++
4	Phenols	+++

The present study revealed the presence of phytoconstituents like Quinine, Tannins, Flavonoids, Phenolic compounds in humic acids. Flavonoid has been referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, antimicrobial and anticancer activities [Aragal *et al.*, 2006]. The antimicrobial activity may be due to the presence of phytochemical constituents like flavonoids and phenolic compounds present in the plant as secondary metabolites [Hosamani PA *et al.*, 2011 & Scalbert A, 1991].

## CONCLUSION

This is to conclude that the humic acid is biologically active, however at typical application rate and the concentration level and the improvement of the each steps which carried before which gives the positive sources of such characteristic methods.

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