

**ANTIMICROBIAL ACTIVITY OF *ASTRAGALUS MEMBRANACEUS* AGAINST DIARRHEAL BACTERIAL PATHOGENS**Balachandar S^{1*}, Jagadeeswari M¹, Dhanabalan R¹ and Meenachi M²¹Department of Microbiology, Rathnavel Subramaniam College of Arts and Science, 242 –B KVK Thottam, Trichy Road, Coimbatore-641 402, Tamilnadu, INDIA²Department of Bioinformatics, Achariya Arts and Science College, Pondicherry-605110, Tamilnadu, INDIA***Corresponding author e-mail:** hellovalu01@gmail.com**ABSTRACT**

Astragalus membranaceus (Fabaceae) is commonly used in medicine to treat a wide variety of infections. However, there is a lack of information on the effectiveness of *A. membranaceus* against microorganisms. The purpose of this study is to determine whether *A. membranaceus* inhibits bacterial growth *in vitro*. Methanolic and Ethanolic extracts of *Astragalus membranaceus* were prepared using dried root and were screened for phytochemical constituents. Tests for alkaloids, Saponins, Terpenoids, Flavonoids and cardiac glycosides were positive in both methanolic and ethanolic extracts. The evaluation of Antimicrobial activity of both the extracts was also carried out. The extracts were tested in disc diffusion assays against Diarrheal bacterial pathogens *Escherichia coli*, *Salmonella enteritidis*, *Shigella*, and *Campylobacter*. The results of antibacterial activity revealed that all the extract showed good inhibitory activity against all the tested pathogens. The activity of the extract was compared with standard antibiotics.

Keywords: *Astragalus membranaceus*, Anti Microbial, Phytochemicals, Medicinal plants, Disc diffusion assay.**INTRODUCTION**

Ayurveda, the ancient Indian therapeutic measure is renowned as one of the major systems of alternative and complementary medicine. As other herbal systems, greater parts of its medicaments are based on indigenous herbals. And the thorough and fractionate knowledge about the medicinal plant is mandatory for all who is working in the field of ayurveda, in order to identify and select the appropriate plant for a specific disease. In the recent years, the interest in medicinal plants has increased in a great deal. Apart from this; people from the west have also taken this matter seriously by conducting various researches on plant based medicines.

The genus *Astragalus* is a very large group of more than 2,000 species distributed worldwide, and is commonly known as milkvetch root. Currently, much of the pharmacological research on *Astragalus* is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immune deficiency

conditions. *Astragalus* has demonstrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. *Astragalus* root has been used to promote immune function and as a tonic to build stamina. *Astragalus* tonifies the lung and is used in cases of frequent colds, spontaneous sweating, and shortness of breath. ^[1] Other traditional indications include wasting disorders, night sweats, chronic ulcerations and sores, numbness and paralysis of the limbs, and edema. ^[2] The main constituents of *Astragalus membranaceus* include polysaccharides, saponins, flavonoids, amino acids, and trace elements. ^[3]

Astragalus contains the plant pigments: formononetin, astraisoflavan, astrapterocarpan, 2'-3'- dihydroxy-7, 4'- dimethoxyisoflavone, and isoliquiritigenin. Other major constituents include D-β-asparagine, calycosin, cycloastragenol, astragalosides IV, VII, choline, betaine, kumatakenin, sucrose, glucuronic acid, β-sitosterol 1, and soyasaponin. ^[4] *Astragalus* has

antibacterial and anti-inflammatory properties. It is sometimes used topically for wounds. In addition, studies have shown that *Astragalus* has antiviral properties and stimulates the immune system. Recent research in China indicates that *astragalus* may offer antioxidant benefits to people with severe forms of heart disease, relieving symptoms and improving heart function. *Astragalus* may also have mild diuretic (rids the body of excess fluid) activity.^[5]

MATERIAL AND METHODS

Plant collection and Phytochemical screening: The plants were collected from the tribal belts of Nilgiris District, Tamil Nadu, India. The plants were identified, confirmed and authenticated by Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu, India. After authentication roots were collected in bulk, washed, shade dried and extracted with methanol and ethanol for 48 hrs in a Soxhlet assembly⁶. The extracts were concentrated, percentage yield calculated and phytochemical screening of the plant extract was carried out to detect the presence of certain bioactive compounds.^[7,8,9]

Test Organisms: Four strains of diarrheal bacterial pathogens - *Escherichia coli*, *Salmonella enteritidis*, *Shigella*, and *Campylobacter* causing diarrhea were used to evaluate the antibacterial activity. These four strains were procured from Department of Microbiology, RVS Hospital, Coimbatore, Tamil Nadu. These organisms were identified by following Standard Microbiological Methods.^[10] All bacterial cultures were maintained in NA slants/ plates; stored at 4°C and periodically sub-cultured.

Antimicrobial Activity Test: Antimicrobial activity was tested using a modified disc diffusion assay (DDA).^[11,12] The inoculum for each microorganism was prepared from broth culture (10⁵ CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm, Hi-media, Mumbai, India) impregnated with the plant extracts (1.0 mg/disc and 5.0 mg/disc) were placed on the cultured plates and incubated for 24 h at 37°C. The solvent

loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

RESULTS AND DISCUSSION

The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts.^[13]

The results of phytochemical screening tests on extracts of root of *Astragalus membranaceus* in different solvents indicated the presence of Alkaloids, Saponins, Terpenoids, Flavonoids and Cardiac Glycosides and the results were depicted in Table 1. Results of antibacterial activity depicts that all the extracts effectively inhibited the growth of *Escherichia coli*, *Salmonella enteritidis*, *Shigella*, and *Campylobacter* with maximum zone of inhibition on the disc loaded with phytochemical extract with different concentrations (1.25, 2.5 and 5 mg/ml/disc). The results of anti-bacterial screening tests in different solvents against pathogenic bacteria using diffusion techniques are depicted in Table 2. Methanolic extract inhibited the growth of all the four tested bacterial strains; however, maximum inhibition zone was recorded against strains namely *E. coli* and *Shigella*. While ethanolic fraction was also found to be effective against all the strains and maximum inhibition was recorded against *Shigella* and *Salmonella enteritidis*.

CONCLUSION

From the results it is clear that roots of *Astragalus membranaceus* are effective against diarrheal pathogens. Methanolic fractions exhibited maximum inhibition followed by ethanolic fractions. These findings suggest new pathway in elucidating a potent antimicrobial agent from *Astragalus membranaceus*. *In vivo* experiments are needed to confirm these findings.

Table 1: Phytochemicals detected in the extracts of *Astragalus membranaceus*

Phytochemicals	Methanolic extracts	Ethanolic extracts
Alkaloids	+	+
Tannins	-	-
Saponins	+	+
Phlobatannins	-	-
Terpenoids	+	+
Flavonoids	+	+
Anthraquinones	-	-
Cardiac glycosides	+	+
Steroids	-	-

Table 2: Antimicrobial activity of root extracts of *Astragalus membranaceus* by disc diffusion method

Organism	Methanolic extract (Zone of Growth inhibition in mm)				Ethanollic extract (Zone of Growth inhibition in mm)			
	C	1.25	2.5	5	C	1.25	2.5	5
<i>Escherichia coli</i>	13	12	18	22	13	-	9	12
<i>Salmonella enteritidis</i>	12	11	15	17	12	-	11	13
<i>Shigella</i>	11	12	16	19	11	-	9	14
<i>Campylobacter</i>	12	8	10	12	12	-	9	11

C - Control; 1.25 - 1.25 mg/disc; 2.5 - 2.5 mg/ disc; 5 - 5 mg/ disc

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