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## Evaluation of antimicrobial activity of *Asclepias curassavica* Ethanol extract

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### ABSTRACT

The present study was carried out for screening of preliminary phyto-chemical constituents and anti-microbial activity of ethanolic extract of ASCLEPIAS CURASSAVICA. The dried extract obtained by soxhlet extraction was subjected to qualitative phyto-chemical screening for the identification. The anti-microbial activity of *A.curassavica* flower & plant extract by agar well diffusion method. Our study reveals that *B.subtilis* & *E.coli* showed good inhibitory zone in ethanol solvent extract

**Keywords:** *Asclepias curassavica*, Phyto-chemical screening, anti-microbial activity, *E.coli*

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## INTRODUCTION

A retrospection of the healing power of plants and a return to natural remedies are absolute need of our time. A modern dictionary defines health as “soundness of physical, mental or moral condition especially freedom from bodily pain or disease.” But true health includes also the joy of living, the power and ability to lead a satisfying and purposeful life.

Health care which was a part of the traditional culture of the people, has become a profession in the modern industrial world. Synthetic drugs created by the affluent and influential pharmaceutical industries have given rise to side effects which are more dangerous than the diseases they claim to cure. The world’s attention has again turned to the traditional medical system.<sup>1</sup>

Plants are an integral part of nature. The physical beauty and its chemistry are of immense importance to humankind.<sup>2</sup> They are the life-sustaining force on earth. Jethro Kloss, a naturopathist, says that there is a wonderful science in nature, in trees, herbs, roots and flowers which man has never fathomed.<sup>3</sup>

He further states that if true remedies are found in nature, then poisonous drugs and chemicals would be eliminated and sickness would be rare.<sup>4</sup>

The medicinal plants assume a tremendous importance, especially at a time when the world is showing a resurgence of interest in the healing properties.<sup>5</sup> The world is endowed with a rich wealth of medicinal plants.<sup>6</sup> Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world as peoples tried to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with the Body’s own defenses.<sup>7</sup> Medicinal plants are economically important as they provide the basic raw materials for pharmaceuticals, phyto-chemical industries. Popularity of herbal medicines is regaining day by day and because of the fact that it has less or no side effects. In the early part of the century, plants were vital sources of raw material for medicines.<sup>8</sup> The efficacy of Indian systems of medicine captured the interest of the elite and modern lines Early man was very close to mother-nature and his observations led to initiate the early research process. By trial and error, very soon a massive pile of information on the medicinal values of the green remedies occurred. Interestingly, plants were, and still are being recorded as the natural drugs or drugs of biological origin for most oral and non-oral medication.<sup>9</sup> And, today’s health care systems will rely largely upon plant material. Plants are composed of different bioactive compounds, which treat numerous ills and disorders. These

active principles are present in plants in variable amount.<sup>10</sup> Therapeutic value and pharmacological action of the drug are due to the presence of a certain chemical constituents viz., various forms of glycosides and alkaloids, derivatives of carbohydrates, gums, resins, volatile oils, pectin and soon in recent years, a lot of progress has been made owing to the availability of highly sensitive technologies.<sup>11</sup>

As a result, chemical composition of certain drug plants was studied upto the molecular level and also the identified chemicals have been screened for pharmaceutical action and mechanism of action which was studied upto the cellular level.<sup>12</sup> Nature has been a source of medicinal agents for thousands of years and a favorable number of modern drugs have been derived from natural sources, and many of these isolations were based on the uses of the agents in traditional medicine.<sup>13</sup>

Medicinal plants are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments for millennia. There is a paradigm shift in universal trend from synthetic to herbal medicine which can be said “Return to Nature”.<sup>14</sup>

Nature has bestowed upon us plenitude of botanical wealth and a large number of diverse types of plants grow wild in various parts of our country. In India the use of different parts of several medicinal plants to cure specific ailments has been prevailing from ancient times.<sup>15</sup>

## MATERIALS AND METHOD

### **Chemicals:**

Nystatine, streptomycin, carrageenan, Diclofenac sodium, conc. hydrochloric acid, sodium hydroxide, acetic anhydride, conc. Sulfuric acid, 2, 4-dinitrophenyl hydrazine, ferric chloride, petroleum ether, chloroform, ethanol, DMSO, Distilled water, Mayer’s reagent, Dragandroff’s reagent, Hager’s reagent, Wagner’s reagent, ferric chloride, magnesium turnings, gelatin in sodium chloride solution, sodium nitro-prusside, pyridine, methanolic alkali, sodium picrate reagent, Molisch’s reagent, Fehling’s reagent, Million’s reagent, copper sulfate solution and iron cyanide.

### ***Collection of plant material:***

The plants *Asclepias curassavica* was collected from near college surrounds and the material was preserved for future purposes and authenticated by botanist.

### **Plant extraction**

The collected plant material of *Asclepias curassavica* was collected near to my college shade

dried, powdered. The powdered plants were sequentially extracted with ethanol. The extracts were air dried using the rotary evaporator.



### **Phytochemical analysis**

Phytochemical analysis of petroleum ether, chloroform and ethanol extracts were carried out for the detection of active secondary metabolite or different constituents such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins and saponins. The dried extracts obtained by soxhlet extraction were subjected to qualitative phytochemical screening for the identification

### ***Anti-microbial activity of A.curassavica flower and plant extracts by agar well diffusion method***

The antifungal and antibacterial screening of the flower extracts and plant extracts were carried out by following the agar well diffusion method (Irobi *et al.*, 1994).The organisms collected were sub cultured. Two wells of 6mm diameter were prepared with the help of a sterile well puncher. The 12 hours culture broth was taken and swabbed over the plate using sterile cotton swabs to obtain a uniform lawn culture. 50mg/mL of the extract was constituted in distilled water and 10% DMSO for the gel

This concentration was introduced into each well and allowed to stand for 30 minutes at room temperature for the proper diffusion. Alongside the solvent control of 10% DMSO and drug positive control Nystatine of 5mg/mL for fungi and drug positive control streptomycin of mg/mL for bacteria were also setup. All the plates were prepared intriplets and incubated at 37°C for 24 hours. After incubation diameters of the inhibition zones were measured and tabulated . The minimum inhibitory concentration (MIC) of the extracts was

determined on the only susceptible organisms. This was investigated by varying the concentration of the extracts.

### Statistical analysis

The experimental data are expressed as mean  $\pm$  SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by students test determine the significant difference between samples with 95% confidence limit.

## RESULTS AND DISCUSSIONS:

### Phytochemicals in plant *Asclepias curassavica*

**Table 1: Preliminary Phytochemicals in plant *Asclepias curassavica*.**

Chemical test for	Ethanol extract
Alkaloids	–
Glycosides	+
Tannins	–
Steroids	+
Flavonoids	+
Saponins	+
Phenols	+
Carbohydrates	–
Proteins	–

‘+’ = *Present*; ‘–’ = *Absent*.

Natural products from plants serve as a vast source of compounds with amazing chemical and functional diversity and make significant contributions to drug development programme. Phytochemicals literally means chemicals produced by plants.

Phytochemicals accumulated in plants play a significant role in the interaction with surroundings. These chemicals are primarily involved in the self-defense of the plants against pests and diseases hence the phytochemicals possess a fighting capacity (fight-o-chemicals). Chemical constituents in plants can be broadly divided into two major groups *viz.*, primary and secondary chemical constituents. The primary constituents are the basic metabolites of plants such as carbohydrates, proteins, lipids, cellulose and chlorophyll which are distributed in almost all the plants. The secondary chemical constituents are selective and vary considerably from plant to plant and even within the species or varieties of the same genus. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. Plants due to their interaction with the environment have evolved secondary pathway that allow them to synthesize an array of chemicals that are duly called as secondary metabolites. These secondary metabolites can be unique to specific species or genera and do

not play role in the plants primary metabolic requirements, but rather they increase their overall ability to survive and overcome local challenges by allowing them to interact with the environment.

Preliminary phytochemical screening was done as an important initial step to determine the phytochemical constituents in *A.curassavica* which helped to make a clear approach towards the objectives of study. Phytochemical analysis showed considerable amount of primary metabolites in *A.curassavica* plant. The secondary metabolites such as alkaloids, saponins, phenolic compounds, steroids, flavonoids, and glycosides were found to be variously distributed in the plant extracts. The results are tabulated in Table.1

Phenolic compounds are secondary metabolites of plants, with different activities such as protection against pathogens and predators, mechanical support, attraction of pollinating animals, and protection against ultraviolet radiation.

Cardiac glycosides are known to work by inhibiting the  $\text{Na}^+/\text{K}^+$  pump. This causes an increase in the level of sodium ions in the myocytes, which then lead to a rise in the level of calcium ions. This inhibition increases the amount of  $\text{Ca}^{2+}$  ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of the heart; thus, they are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also used to strengthen a weakened heart and allow it to function more efficiently though the dosage must be controlled carefully, since the therapeutic dose is close to the toxic dose .

The therapeutic potential of these flavonoids have been determined and are known to have a number of pharmacological and biochemical properties namely antibacterial, antiviral, anti-allergic, vasodilatory and anti-inflammatory exhibiting activity against the enzymes cyclooxygenase and lipoxygenase. Flavonoids also exert the effects of anti-oxidants, free radical scavengers and are chelators of divalent cations.

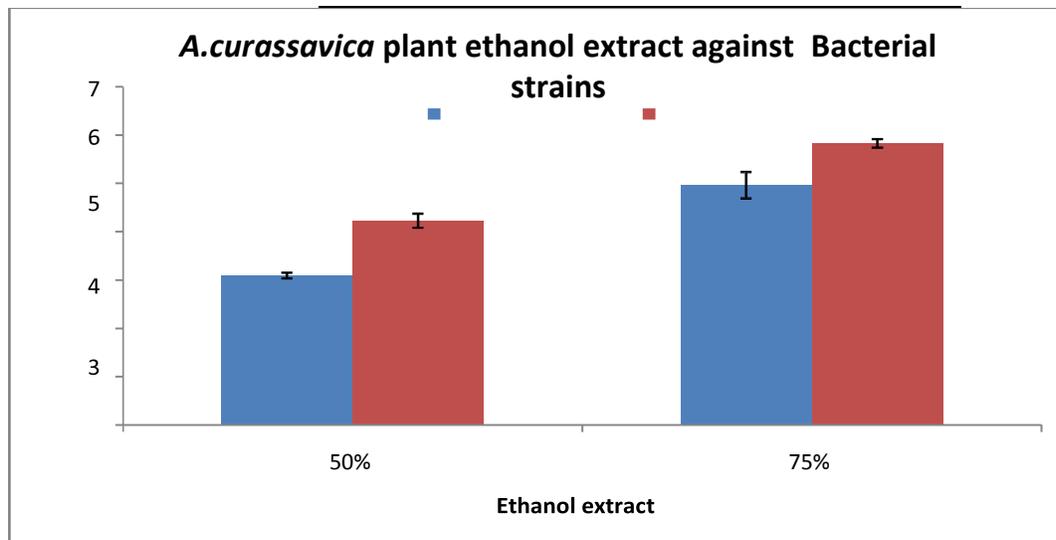
**Table 2: *A.curassavica* plant extracts on different bacterial species**

<b>Name of the organism</b>	<b>Ethanol extract</b>
<i>Bacillus subtilis</i>	+
<i>E.coli</i>	+
<i>Proteus Vulgaris</i>	
<i>Shigella flexheri</i>	-
<i>Salmonella typhimurium</i>	

Each value is the average diameter of triplicates, '+' – inhibition; '-' – No inhibition.

**Table 3: Determination of MIC of *A.curassavica* plant extract on different Bacterial species.**

Names of the organism	Ethanol extract	
	50mg/mL	75mg/MI
Ethanol control	00	00
<i>Staphylococcus aureus</i>	0.00	0.00
<i>Shigella flexheri</i>	0.00	0.00
<i>Salmonella typhimurium</i>	0.00	0.00
<i>Bacillus subtilis</i>	3.10±0.05	4.97±0.27
<i>E.coli</i>	4.23±0.14	5.83±0.08



**Figure 2: The Zone of Inhibition of the ethanol extract of *A. curassavica* on different Bacterial species.**

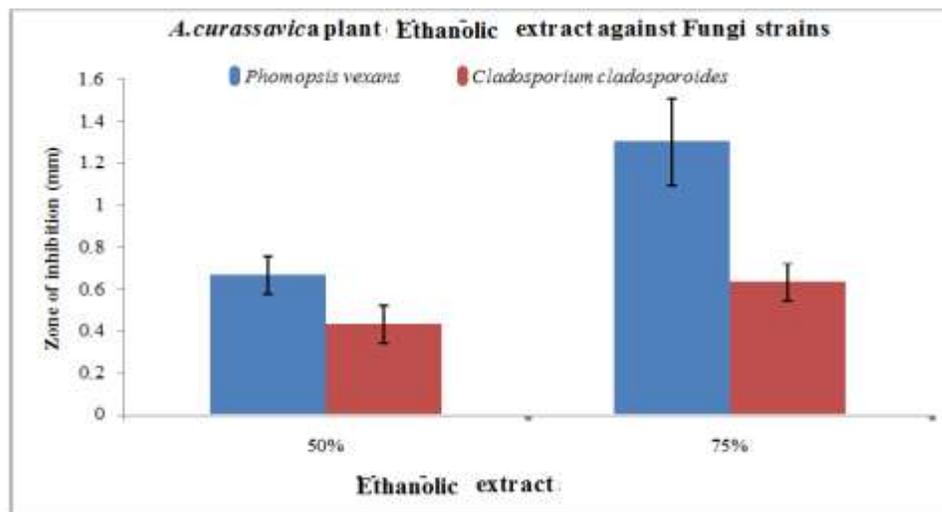
**Table 4: *A.curassavica* plant extracts on different Fungal species.**

Names of the	Ethanol extract
<i>Aspergillus niger</i>	—
<i>A.flavus</i>	—
<i>Fusarium</i>	—
<i>F.oxysporum</i>	—
<i>Alternaria alternate</i>	—
<i>Phomopsis vexans</i>	—
<i>Cladosporium</i>	—

**Table 5 Determination of MIC of *A.curassavica* plant extract on different fungal species.**

Names of the organism	Ethanol extract	
	50mg/mL	75mg/MI
Ethanol control	00	00
<i>Aspergillus niger</i>	0.00	0.00
<i>A.flavus</i>	0.00	0.00
<i>Fusarium sporotrichioides</i>	0.00	0.00
<i>F.oxysporum</i>	0.00	0.00

<i>Alternaria alternata</i>	0.00	0.00
<i>Phomopsis vexans</i>	0.00	0.00
<i>Cladosporium cladosporoides</i>	0.00	0.00



**Figure 3: The Zone of Inhibition of the Ethanolic extract of *A. curassavica* on different fungal species.**

## CONCLUSION

Plants have been an essential part of human society since the civilization started. They form the basis of traditional medicine systems. Medicinal plants play a vital role in drug discovery and are very useful for human to cure various ailments. There is a worldwide interest in searching for the safe new phytochemical compound drugs. There is a vast wealth of medicinal plants that have not been explored and exploited in which they contain bioactive constituents that could be exploited for human welfare. In this contextual evaluation of plant extracts of antimicrobial activities were taken up to accomplish the desired objectives of the current studies. The present thesis encompasses the results and discussion of these studies and summarized below.

- General introduction to the medicinal plants and their uses.
- Ethno botanical enriched knowledge and of their traditional uses in India.
- The holistic approach in medical system.
- A review on various medicinal plants with their anti-microbial activities.
- A brief overview on selected bacteria and fungus.
- Literature review on ethano medical, pharmacological, and phyto constituents of selected plants such as *Asclepias curassavica*

The plant *Asclepia scurassavica* was collected from college surroundings. The specimen sample was identified and authenticated by the our college department of pharmacognosy, Howrah. Plant material was shade dried and powdered and successively extracted with ethanol solvent by using the soxhlet apparatus. The extracts were dried using the rotary vacuum evaporator.

Phytochemical analysis showed the presence of the secondary metabolites such as alkaloids, saponins, phenolic compounds, steroids, flavonoids, and glycosides was found to be variously distributed in the plant extracts When plant extracts of *A. curassavica* was tested for antimicrobial activity, the result revealed that *B.subtilis* and *E.coli* showed good inhibitory zone in ethanol solvent extract. Whereas, *Proteus vulgaris*, *Shigella flexheri* and *Salmonella typhimurium* were found to be resistant and remained unaffected by all the solvent extracts.

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