

# *In Vitro* Evaluation of Anti Bacterial Activity Three Herbal Extracts on Methicillin Resistant Staphylococcus aureus [MRSA]

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## Abstract

**Objective:** The aim of the present study was to assess the anti bacterial activity of ethanolic extract of *Heart wood of Acacia catechu willd*, *Aesculus hippocastanum*, *Glycyrrhiza glabra*. These extracts are the oldest known herbal remedies famous for its vast variety of therapeutic properties. Ethanolic extract of these herbs were tested for antibacterial activity against multi drug resistant *Staphylococcus aureus* [MRSA]

**Method:** The screening of antibacterial activity of the three selected plant extracts was carried out using the agar well diffusion method. The wells were loaded with 100µl of the three herbal extracts at different concentrations [500ug/disc, 1000ug/disc and 2000µg/disc]. Positive controls used were standard antibiotic discs of amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc)

**Result :** After incubation at 37°C for 24 hours, the zone of Inhibition was measured .

**Conclusion:** The extracts at different concentrations showed varying degree of antibacterial activity against the micro organisms tested compared to standard.

**Key Words:** *Acacia catechu willd*, *Aesculus hippocastanum* *Glycyrrhiza glabra*, disc diffusion, zone of inhibition, anti bacterial activity.

## INTRODUCTION

The expanding bacterial resistance to antibiotics has become a growing concern world wide. The rise in antibiotic resistance has resulted in a decreasing number of fully active antimicrobial agents available to treat infections caused by multi-drug resistant (MDR) bacteria. Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients.<sup>1</sup> *Staphylococcus aureus* is an important cause of serious infections in both hospitals and the community. Methicillin-resistant *S. aureus* (MRSA) includes those strains that have acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics. MRSA was first reported in 1961, soon after methicillin was introduced into human medicine to treat penicillin resistant staphylococci. This group of organisms has emerged as a serious concern in human medicine. Although these organisms cause the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenging.<sup>2</sup> This has necessitated a search for new antimicrobial agents. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds. Plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, alkaloids, and flavonoids, which are reported to have in vitro antibacterial properties.<sup>3</sup> Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat. Herbal remedies may offer novel treatment options which elicit little or no transferred resistance if used in optimal concentrations.<sup>4</sup>

*Acacia catechu* (AC) (Family: Fabaceae and subfamily: Mimosoideae) known as Black cutch.AC is medium sized thorny deciduous tree mainly found in India. It is said that the

name 'catechu' was given to it because its bristles resemble the claws of animals of the cat family or may be because its heart wood contains gummy extract called kath or catch.<sup>5</sup> The chief constituents of the heartwood are catechin and catechutannic acid . The wood contains epicatechin , Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercitrin, quercitin<sup>6</sup>. Catechin is biologically highly active. The extract of *Acacia catechu* extract have been reported to have various pharmacological effects like antibacterial,<sup>7</sup> anti oxidant<sup>8</sup>, immuno modulatory<sup>9</sup>, anti pyretic , hypoglycaemic, anti diarrhoeal hepatoprotective activity.<sup>10</sup>

*Aesculus hippocastanum* (family Hippocastanaceae) commonly known as Horse chestnut is native to Western Asia. Horse chest nut is also known as Spanish chestnut, buckeye, seven leaves tree, is a deciduous tree up to 35 meters high.<sup>11</sup> Active Chemical Constituents of horse chest nut are coumarin derivatives like aesculin, fraxin, scopolin; flavonoids like quercetin, kaempferol, astragalol, isoquercitrin, rutin, leucocyanidine and essential oils like oleic acid, linoleic acid.<sup>12</sup> The seeds have been used as an analgesic, antipyretic, narcotic, tonic, and vasoconstrictor. They have been used to treat backache, sunburn, neuralgia, rheumatism, whooping cough and hemorrhoids.<sup>13</sup> It has antilipemic, expectorant, diuretic properties and antimicrobial activity. It is also used for the prevention of gastric ulcers, reduction of cerebral edema, reduction of cellulite, as adrenal stimulant, hypoglycemic agent, antithrombotic, anti-inflammatory, and also for reduction of hematomas and inflammation from trauma or surgery.<sup>14</sup>

*Glycyrrhiza glabra*,( family Fabaceae) also known as liquorice and sweet wood, is native to the Mediterranean and

certain areas of Asia. It is a perennial herb which possesses sweet taste.<sup>15</sup> The herb contains glycyrrhizin, glycyrrhetic acid, flavonoids, asparagine, iso-flavonoids, and chalcones.<sup>16</sup> The glycoside, glycyrrhizin has a similar structure and activity as the adrenal steroids. Licorice also acts like the hormone, ACTH, causing sodium retention, potassium depletion, and water retention. It also possess good anti bacterial,<sup>17</sup> anti fungal,<sup>18</sup> anti oxidant,<sup>19</sup> antitussive,<sup>20</sup> hepatoprotective<sup>21</sup> and anti inflammatory activity.<sup>22</sup> Historically, the dried rhizome and root of this plant were employed medicinally as an expectorant and carminative. It is used for treating upper respiratory ailments including coughs, hoarseness, sore throat.

## MATERIALS AND METHODS

### Plant material

The ethanolic extract of *Heart wood of Acacia catechu willd*, *Aesculus hippocastanum*, *Glycyrrhiza glabra* was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

### Test microorganisms

Bacterial strains used is multidrug resistant *Staphylococcus aureus*[MRSA]. The organisms was obtained from department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

### Methodology

The extracts were prepared in the following concentrations in sterile water. 5mg/ml and 10mg/ml and 20mg/ml. so that 100µl of extract of different concentrations delivers 500µg, 1000 µg and 2000µg respectively.

### Assay for antibacterial activity using agar well diffusion method

The screening of antibacterial activity of the three selected plant extracts was carried out using the agar well diffusion method. The bacterial strain was inoculated into nutrient broth and incubated at 37°C overnight. The culture was then adjusted to 0.5 McFarland turbidity standard.<sup>23-26</sup> Lawn culture of the test organism was made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. A sterile cork borer was then used to make wells (6mm diameter) for different concentrations of the extracts 100µl of the varying concentrations (500,1000, 2000µg) of the extracts were introduced into the wells with the help of micropipettes. The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of amoxicillin (30mcg/disc) and Ciprofloxacin (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

## RESULT AND DISCUSSION

The antibacterial activity of the extracts at different concentrations was screened by agar well diffusion technique

and the zone of inhibition was measured in mm diameter. The results are given in the table 1.

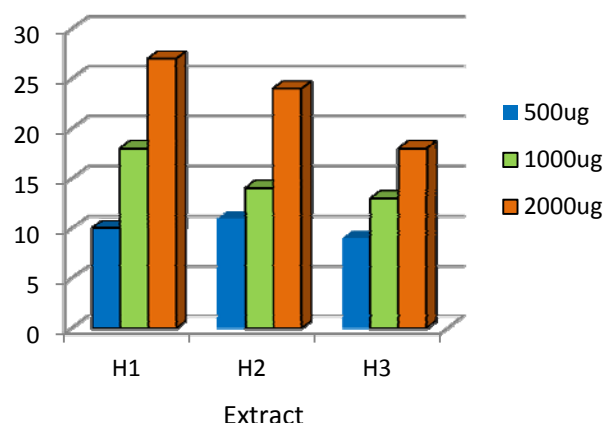
The extracts at different concentration exhibited antibacterial activity against the bacterial strain tested. The zone diameter increased with increase in concentration of the extracts. The Heart wood extract of *Acacia catechu willd* was more effective against and multi drug resistant *Staphylococcus aureus* with a zone of inhibition of 27mm diameter (at conc 2000 µg.) and *Aesculus hippocastanum* was less effective with zone of inhibition of 18mm (at conc. 200 µg.). *Glycyrrhiza glabra* showed a zone of inhibition of 24mm diameter (at conc. 2000 µg)

**Table 1 :** Anti bacterial activity of plant extracts on Multi drug resistant *Staphylococcus aureus*

Extracts	Conc [µg]	Zone of inhibition [in mm diameter]		
		H1	H2	H3
Extracts	500	10	11	09
	1000	18	14	13
	2000	27	24	18
<i>Ciprofloxacin</i>	30mcg/disc	24	21	22
<i>Amoxicillin</i>	30mcg/disc	25	23	25

H1- *Heart wood of Acacia catechu willd*, H2 -*Aesculus hippocastanum*, H3-*Glycyrrhiza glabra*,

**Graph 1 :** Anti bacterial activity of plant extracts on Multi drug resistant *Staphylococcus aureus*



H1- *Heart wood of Acacia catechu willd*, H2 -*Aesculus hippocastanum*, H3-*Glycyrrhiza glabra*,

Prevalence of drug resistance to various antibiotics among pathogenic bacteria has paved way for the search of new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases

than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens.

The present study was to evaluate the antibacterial activity of the three herbal extracts - *Heart wood of Acacia catechu willd*, *Aesculus hippocastanum*, *Glycyrrhiza glabra* against MRSA. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

#### CONCLUSION

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established. It is clear from the results that, the extracts acts as a good source of antimicrobial agent against drug resistant *Staphylococcus aureus*.

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