Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/4239
DOI URL: http://dx.doi.org/10.21474/IJAR01/4239
ISSN NO. 2320-5407

RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *CISSUS ARISTATA* BLUME AGAINST HUMAN PATHOGENIC BACTERIA.

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Manuscript Info

Manuscript History

Received: 21 March 2017
Final Accepted: 24 April 2017
Published: May 2017

Key words:-
Cissus aristata, antibacterial analysis, disc-diffusion method.

Abstract

The antibacterial activity on dried root and stem of *Cissus aristata* were evaluated on strains like *Klebsiella pneumoniae* and *Staphylococcus aureus.* Petroleum ether, chloroform and ethanol were used for the present study. The invitro antibacterial activity was performed by agar disc diffusion method. The study reveals that the plant extracts showed varying degree of antibacterial activities against these bacterial strains. The activities of these three extracts were compared with that of one standard antibiotic (Gentamicin). Stem extracts are highly significant against *Staphylococcus aureus* and not against *Klebsiella pneumoniae*. Chloroform root extract is significant against *Klebsiella pneumoniae* but not against *Staphylococcus aureus.* A preliminary phytochemical screening was conducted on the plant extracts using standard qualitative procedures that revealed the presence of the secondary metabolites like alkaloids, tannins (condensed), flavanoids, phytosterols, triterpenoid, lactones, volatile oil and saponin.

Introduction:-

The plant have fed the world and cured its ills since time immemorial (Parikh K. M, 1991). For many centuries, humans have relied on plants for survival and pleasure (Randy Moore *et al*., 1998). From the earliest times man had to distinguish between those plants which were poisonous and those which were not and there gradually developed the knowledge of naturally occurring drugs (George Edward *et al*,. 1972). The study of medicinal plant is a highly interdisciplinary science, chemical, biochemical, pharmaceutical and biological properties of drugs or drug substances of natural origin as well as the search for new drugs from natural sources. The medicinal importance of a plant is due to the presence of complex secondary metabolites like alkaloids, glycosides, resins, volatile oil, gums, tannins, flavanoids, fixed oil, etc. These active principles usually remain concentrated in the storage organs of the plant's leaves, roots, seeds (Arvind Kumar, 2011).

Vitaceae (the great family) according to Hooker's classification consisted of a single genus vitis and considered to be economically important.The species of this family shows many medicinal properties like antiulcer activity, anxiolytic, anticonvulsant, antiradical and antibacterial activity. They are also used for the treatment of several diseases such as rheumatism, epilepsy, stroke and also in the treatment of diabetes. *Cissus aristata* Blume, a member of Vitaceae is selected for the current study.

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Materials and Methods:-

Collection of plant material:-

During the field trip conducted for taxonomical collection the plant *Cissus aristata* Blume was collected from hilly regions of Mangad, Thrissur. And it's identity was confirmed by referring relevant literature.

Plant extraction Method:-

Shade dried stem and root powder weighing 10g and 3g respectively of *Cissus aristata* were extracted sequentially with 300ml of solvents namely petroleum ether, chloroform, and ethanol. The extracts were filtered & concentrated using vaccum distillation, under high pressure at 110°C. It is then subjected to screen the phyto chemicals and antibacterial assays.

Phytochemical screening:-

The different extracts were subjected to preliminary phytochemical screening for the determination of major chemical groups by standard procedures (Kokate C. K, 1990).

Micro organisms used:-

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Infectious diseases caused by the microbes are major health hazards all over the world. The control of our infectious diseases has been the greatest achievement of medical science.

Special features of selected microorganisms:-

Klebsiella pneumoniae
Order:Enterobacteriales
Family: Enterobacteriaceae

It is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum. *K. pneumoniae* can cause destructive changes to human lungs via inflammation and haemorrhage with cell death(necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum). *Klebsiella* infections are seen mostly in people with a weakened immune system.

Staphylococcus aureus
Order : Eubacteriales
Family : Micrococcaceae

It is a Gram-positive, non motile, coccus, occurring singly, in pairs, short chains, or in irregular clusters. It is a spherical bacterium frequently part of the skin flora found in the nose and on skin. *Staphylococcus aureus* are becoming increasingly resistant to many commonly used antibiotics including pencillins, macrolides, such as erythromycin, tetracyclines and aminoglycosides. They cause skin obsesses, wound infection, pneumonia and other systematic infections (Michael J. P, 1986)

Antibacterial Assay by Disc Diffusion Method:-

Plant extracts of stem and root of *Cissus aristata* were prepared with different solvents like petroleum ether, chloroform, ethanol. Antibacterial activity was demonstrated using a modification of the method originally described by which is widely used for the antibacterial susceptibility testing. Liquid nutrient agar media and the petriplates were sterilized by autoclaving at 120°C for 30 minutes. A spirit lamp is also kept lightened. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each petriplate to yield a uniform depth of 4 mm. After solidification of the media, the bacterial strains of *K. pneumoniae*, *S. aureus*, were swabbed using cotton swabs in an aseptic condition on the surface of the agar plates. All regions are swabbed uniformly. Except the antibiotic disc, the paper disc are dipped in the extracts. All the petri plates were labeled correctly. Whatsmann No.1 filter paper disc was autoclaved. These discs were dipped into each 3extracts of stem and root. The prepared discs of plant extracts, antibiotics were kept at equal distance on the inoculated nutrient agar plates using a clean sterile forceps. At each and every time the tip of the forceps must be flamed in order to kill the microorganism. Triplicates were placed for each and every experiment. Then all the

petriplates incubated at 37°C for 24hours, the antibacterial drug which was used here was Gentamicin for standard. Then after a day observe the plates. Pure solvent used as negative control.

Results and Discussion:-

17 tests were carried out to identify the secondary metabolites. Among that 9 tests shows positive results in various extracts. The secondary metabolites such as alkaloids, tannins(condensed), flavanoids, phytosterols etc., are present in ethanol stem extract. Tanins are present in the ethanol root extract. Saponin is present in the chloroform extract and petroleum ether root extract. Triterpenoid and lactones are present in the petroleum ether stem extract. Volatile oil is present in the chloroform stem and root extract. (Table 1)

Table 1:- Showing the phytochemical analysis of stem and root extracts of *Cissus aristata* using different chemical reagents.◦

	Stem P.E	Stem Chloroform	Stem Ethnol	Root P.E	Root Chloroform	Root Ethanol
Alkaloids	-	-	+	-	-	-
Tannins (Condensed tannin)	-	-	+	-	-	+
Flavanoids	-	-	+	-	-	-
Phytosterol	-	+	+	-	-	-
Phenols	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Saponin	-	+	-	+	-	-
Coumarins	-	-	-	+	-	-
Resin	-	-	-	-	-	-
Triterpenoid (steroid)	+	-	-	-	-	-
Lactones	+	-	-	-	-	-
Volatile oil	-	+	-	-	+	-
Naphthoqunons	-	-	-	-	-	-
Sterol	-	-	-	-	-	-
Carbohydrate	-	-	-	-	-	-
Polysacccharides	-	-	-	-	-	-

+ indicates presence and – indicates absence.

The antibacterial activity of the stem and root of *C.aristata* against two bacteria in petroleum ether, chloroform and ethanol were screened. Plant extracts showed varying degree of antibacterial activities against these bacterial strains. The activities of these three extracts were compared with that of one standard antibiotic (Gentamicin). Stem extracts are highly significant against *S. aureus* and not against *K. pneumoniae*. Chloroform root extract is significant against *K. pneumoniae* but not against *S.aureus.* The tables below shows the activity of each bacterium against different plant extracts.

Table 2:- Antibacterial activity of various extracts of *C.aristata* root against *Klebsiella pneumoniae.*

Sl.No.	Sample	Diameter of inhibition zone (mm)			Average diameter of the zone mm)	Negative control	Actual diameter of zone (sample-control)
		Exp.1	Exp.2	Exp.3			
1	Petroleum ether	0	0	0	0	7	0
2	Chloroform	17	15	16	15	6	9
3	Ethanol	0	0	0	0	9	0
4	Gentamicin	18	16	17	17	-	-

In the case of *C. aristata* root against *Klebsiella pneumoniae* only the chloroform shows inhibition against the bacteria. Other extracts has no activity (Table 2).

Table 3:- Antibacterial activity of various extracts of *C.aristata* stem against *Klebsiella pneumoniae.*

Sl.No.	Sample	Diameter of inhibition zone (mm)	Average diameter of the	Negative control	Actual diameter of

		Exp.1	Exp.2	Exp.3	zone mm)		zone
1	Petroleum ether	0	0	0	0	7	0
2	Chloroform	0	0	0	0	6	0
3	Ethanol	0	0	0	0	9	0
4	Gentamicin	18	16	17	17	-	-

In the case of stem extracts of *C. aristata* against *Klebsiella pneumoniae* none of the extracts shows activity (Table 3).

Table 4:- Antibacterial activity of various extracts of *C.aristata* stem against *Staphylococcus aureus*

Sl.No.	Sample	Diameter of inhibition zone (mm)			Average diameter of the zone mm)	Negative control	Actual diameter of zone (sample-control)
		Exp.1	Exp.2	Exp.3			
1	Petroleum ether	11	10	12	11	0	11
2	Chloroform	16	15	14	15	0	15
3	Ethanol	9	7	8	8	0	8
4	Gentamicin	14	13	15	14	-	-

In the case of *C. aristata* stem against *Staphylococcus aureus* it shows activity in all the 3 extracts. Petroleum ether shows a zone of 11mm, chloroform shows a zone of 15mm and ethanol shows a zone of 8mm (Table 4).

Table 5:- Antibacterial activity of various extracts of *C.aristata* root against *Staphylococcus aureus.*

Sl.No.	Sample	Diameter of inhibition zone (mm)			Average diameter of the zone mm)	Negative control	Actual diameter of zone
		Exp.1	Exp.2	Exp.3			
1	Petroleum ether	0	0	0	0	0	0
2	Chloroform	0	0	0	0	0	0
3	Ethanol	0	0	0	0	0	0
4	Gentamicin	15	13	14	14	-	-

In the case of *C.aristata* root against *Staphylococcus aureus* no extracts shows activity(Table 5).

Negative controls were also used. Then the zone formed by negative control is substracted from the zone produced by plant extracts to find the actual activity of plant extracts. Chloroform produces maximum zone of inhibition 15mm against S.aureus in stem and 9mm against *K.pneumoniae* in root. Petroleum ether produces 11mm inhibition zone and ethanol produces 8mm inhibition zone against S.aureus in stem and no activity in others. Gentamicin standard antibiotic produces 18mm inhibition zone in root extract against in *K.pneumoniae* and 14mm inhibition zone in stem extract against *S.aureus* (Table 2-5).

Antibacterial activity was shown in *Klebsiella pneumoniae* by the chloroform root extract of *C. aristata* and in *Staphylococcus aureus* by the petroleum ether, chloroform and ethanol stem extracts of *C. aristata*. From this it is understood that the root extracts can be used as an antibacterial drug against gram negative bacteria because *K. pneumoniae* is a gram negative bacteria. Stem extract has no effect against gram negative bacteria. And in contrary stem extracts can be used as an antibacterial drug against gram positive bacteria since *S. aureus* is a gram positive bacterium. But root extracts has no effect against gram positive bacteria.

Conclusion:-

The result of the present study supports the protection and conservation of *Cissus aristata* which is a rare plant. The study also reveals the fact that, use of this natural drug in the optimum dosage can replace the use of antibiotics.

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