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STUDIES ON PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ROOT OF ACONITUM FEROX WALL (VATSANABH)

Mangesh B. Thakre[1], Akshay N. Patil[2], Nilesh Gandhare[2], Sudhanshu Kharkate[1], Sandeep Hate[1],Priya Thakre[1], Rakesh Naktode[1].

[1]D. R. B. Sindhu Mahavidyalaya, Nagpur. 440017,India.

[2]Nabira Mahavidyalaya, Katol, Dist.Nagpur. India.

mangeshthakre@gmail.com

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ABSTRACT:

The medicinal plant Aconitum ferox wall from Katol region district Nagpur (MS) India was collected. The shade dried roots of Aconitum ferox wall was grind well into fine powder in mixture grinder. The powdered plant material was extracted using soxhlet apparatus with organic solvents like ethanol, chloroform, ethyl acetate, methyl acetate, acetone etc. Phytochemical screening of this plants was performed for Alkaloids, Glycosides, Phenol, Terpenoid, Phlobatanins, Anthraqinone, Flavonoids, Tannin, Saponins, Steroids and carbohydrate. In Vitro antimicrobial and antifungal activities were examined for alcohol extracts. Antibacterial were of plant part extracts against six pathogenic bacteria and one pathogenic fungi were investigated by the paper disc diffusion method by using Mueller-Hinton medium.

Keywords: Medicinal plants, Phytochemical, Antimicrobial activity, Antifungal activity.

INTRODUCTION:

All over the world plants were used as main sources of medicine by human kind. The rise of modern western medicine was initially accompanied by a decline in the practice of herbalism in all cultures and we started believing that synthetic chemical were the best medicines to treat illness and cure disease [1]. Medicinal plants are called medicinal herbs, have been discovered and used in traditional medicinal practices since prehistoric times. All plants synthesis hundreds of chemical compounds for function including defense against insects, fungi, diseases and herbivorous mammals. All plants produce chemical compound which given them an evolutionary advantage such as defending against herbivores. These phytochemicals have potential for use as drugs and the content and known pharmacological activity of these substances in the medicinal plants in the scientific basis for their use in modern medicine if scientifically confirmed [2]. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anesthetic agents and are found in medicinal plants [3].The Aconitum is genus of over 250 species of flowering plants belonging to the family Ranuculaceae. These herbaceous perennial plants are chiefly native to the mountainous parts of northern hemisphere [4].*Aconitum ferox* is a member of monkshood genus Aconitum of Rananculaceae. *Aconitum Ferox* is also called as

"Monk's Hood". It is a deciduous perennial herb with tall and erect stems crowned by racemes of large eye-catching blue, purple, white zygomorphic flowers with numerous stamens [5].

In ancient times, vaidhya used to treat patients on individual basis, and prepare drug according to the requirement of the patient. Herbalism is traditional medicinal or folk medicine practice based on the use of plants and plant extracts [6].Plants have a long-lasting history in many indigenous communities and continue to provide useful tools for treating various diseases. A large number of country's rural population depends on medicinal plants for Medicinal treating various illness. These plants played a significant role in various ancient traditional system of medication in India [7].

Plant based medicines have been used by mankind since time immemorial. According to the report of World Health Organization (WHO), over 80 % of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care [9].

Aconitum ferox wall (Vatsanabha) has been classified under the Sthavaravisa (vegetable poison) group of drugs.The dried root *Aconitum ferox* wall is alterative, anesthetic, antiarthritic, deobstruent, diaphoretic, diuretic, sedative, stimulant. It has been used in India and Nepal in the cure of neuralgia, leprosy, fevers, cholera and rheumatism.

An antimicrobial is agents that kills the micro-organisms or stop their growth [10].

Antibacterial agents can further subdivide into bactericidal agents, who kill bacteria and bacteriostatic agent, which slow down bacterial growth [11].

Antibacterial are used to treat bacterial infections. The drug toxicity to human and other animal from antibacterial is generally considered low [12]. Antifungal are used to kill or prevent further growth of fungi.

Alkaloids:

Wang *et al.*, [2006] ; Pelltier *et al.*, [1968] carried out qualitative phytochemical screening of tuberous roots of genus Aconitum revealed that it contains alkaloids benzoylmecasonine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine and [Wang et al., 2006] plant contain alkaloids: heteratisine, heterophyllisine, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine [15-16].

Murayama *etal.*,[1991]; Oyama *etal.*,[1994]; Amer i,[1998]; Taki *etal.*,[1998]investigated that the tubers of the *Aconitum* species have been used as herbal drug only after it was treated by immersion in salt solution or by heating to reduce the toxicity. Currently the processed aconite tubers are widely and safely used for the treatment of pain neuronal disorders and inflammation with no problematic or annoying adverse effects [17-20].

In one of the study by Jiang *et al.* [2005], three *Aconitum* alkaloids (aconitine, mesaconitine and hypaconitine) in processed and unprocessed tubers were separated by modified HPLC method employing a C18 column gradient eluted with acetonitrile and ammonium bicarbonate buffer. Processed tubers showed lower level of alkaloidal content as compare to unprocessed tubers. The variations obtained were attributed to differences in species, processing methods and places of origin [21].

Flavanoids:-

In 1981 Bisset, explained that the genus *Aconitum* comprises of many European and Asian species. Lim *et al.*, [1999] flavonoids studied in the last ten years as chemotaxonomic markers [22-23].Koes *et al.*, [1994] found that they give color to flowers which attracts the pollinating animals. Besides pigmentation, flavonoids also exhibit phenomenon of co-pigmentation which imparts different shades to the flower [25]. Treutter, [2005] investigated that Flavonoids are mainly involved in photo protection from sunlight ultraviolet and are also potent scavengers of reactive oxygen species which prevent lipid peroxidation [24].

Neurological studies:-

In 1998, Ameri carried out the work research on Effect of several *Aconitum* alkaloids on central nervous system was screened by after dividing them in three different groups comprising of highly toxic, less toxic and reduced toxic alkaloids on the basis of their structure. The pharmacology and therapeutic v/s toxic potential of these alkaloids has been highlighted and discussed which is of great importance [19].

MATERIALS AND METHODS

3.1 Plant collection:-

The present work was carried out at the Department of Chemistry, Nabira Mahavidyalaya, Katol, Nagpur. The plant was selected on the basis of morphology from Wagh Brothers Ayurvedic Medicine shop, Itwari, Nagpur, Maharashtra. The plant was identified and authenticated in Department of Botany, Nabira Mahavidyalaya, Katol, Nagpur. The Roots of *Aconitum ferox wall* was washed thoroughly with water and dried under the shade for about 10-12 days. The dried plant sample was grind well into fine powder in mixture grinder.

3.2 Preparation of Extract:-

The powdered plant material was extracted using soxhlet apparatus with organic solvents like ethanol, chloroform, ethyl acetate, methyl acetate, acetone etc.

3.3Preliminary Phytochemical Screening:-

The extract of *Aconitum ferox wall* was screened for the presence of phytochemical constituents such as Alkaloids, Glycosides, Phenol, Terpenoid, Phlobatanins, Anthraqinone, Flavonoids, Tannin, Saponins, Steroids and carbohydrate following standard procedure.

3.4 Phytochemical Analysis:-

Phytochemical screenings were performed using standard procedure.

Test for Alkaloids (Wagner's Test):-To about 3ml of sample solution, few drops of Wagner's reagent are added. Brownish precipitate is indicates the presence of Alkaloids.

Test for Glycosides:-2ml of acetic acid, 2ml of chloroform with whole aqueous plant crude extract, cool mixture and add concentrated H2SO4acid, green colour show entity of aglycone, steroidal part of glycoside.

Test for Phenols:- To the extract 1-2 ml of neutral $FeCl_3$ was added appearance of violet colour indicates the presence phenol.

Test for Saponins:-In a test tube containing 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 mins. Formation of honeycomb like forth indicates the presence of saponins.

Test for Tannins:-Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and $FeCl_3$ was added to filtrate. A dark green solution indicates the presence of tannins.

Test for Flavonoids:-Extract of about 0.2 gm was dissolved in dilute NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

Test for Terpenoids:- To the extract 2 ml of chloroform and concentrate H_2SO_4 was added slowly to form a layer of reddish brown colouration indicates the presence of terpenoids.

Test for Phobatannins:-To the extract 2% HCl solution was added, formation of red precipitate indicates the presence of phobatannins.

Test for Anthraquinones:- About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allows cooling. Equal volume of $CHCl_3$ was added to the filtrated. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink color indicates the presence of anthraquinone.

The Antimicrobial Activity Screening:-

In Vitro antimicrobial and antifungal activities were examined for alcohol extracts. Antibacterial were of plant part extracts against six pathogenic bacteria (two is gram positive and three is gram negative) and one pathogenic fungi were investigated by the paper disc diffusion (kieby and baur) method by using Mueller-Hinton medium. A total 6 microbial strain used to observe the antibacterial and antifungal activity of *Aconitium ferox wall.* (Table No. 1).

All the strain was procured from NCIM, Pune. Make their sub culturing in a nutrient agar slant and nutrient broth. Incubate them at 37°C for bacteria and fungi was grown in potato dextrose, agar or Subouraud dextrose agar respectively at 28°C and stock culture maintained at 4°C.

Preparation of Inoculum

The bacterial stock cultures were preserved at 4°C on nutrient agar slant. Whereas the fungi were grown in subouraud dextrose agar and PDA media maintained at 28°C.The stock culture were maintained at 4°C. Inoculum suspension were prepared from fresh, mature (3 to 5 days old) culture grown on sabouraud agar or potato dextrose agar slants. The colonies were covered with 5ml of sterile distilled water. 20ml (5%) was added to facillet the precaution of *Aspergillus inoculla* [26]. The active Culture for the experiment were prepared by transforming a loopful of Mueller Hinton broth (oxoid Englant) for bacteria and they are incubated without without agitation for 24Hrs at 37°C.

RESULTS AND DISCUSSIONS

From the Table No. 8 it is clear that,

1. Alkaloids:-It was found that concentration of alkaloid has been extracted in ethanol, chloroform, methyl acetate, ethyl acetate and distilled water extract.
2. Glycosides:-All the extract shown negative test for Glycosides.
3. Phenol:-It was found that concentration of phenol has been extracted in ethanol, methyl acetate and distilled water extract.
4. Saponins:-It was found that concentration of saponins has been extracted in ethanol, chloroform and ethyl acetate extract.
5. Tannin:-All the extract shown negative test for Tannins.
6. Flavonoids :-It was found that concentration of flavonoids have been extractred in ethanol, acetone and distilled water.
7. Terpenoids :-It was found that concentration of terpenoids have been extracted in ethanol and methyl acetate extract.
8. Phobatannins :-All the extract shown negative test for phobatannins.
9. Anthraquinones :-All the extract shown negative test for Anthraquinones.
10. Carbohydrates:-It was found that concentration of carbohydrates have been found in Ethanol and methyl acetate extract**.**

4.2 MICROBIAL ACTIVITY

In a present study an extract obtained from root of *Aconitium ferox wall* plant apply for the antimicrobial activity findings are as follow in table 9.

The present study zone of inhibition was analyzed using ethanol extract of *Aconitum ferox wall* used for antibacterial and antifungal activity, it showed that 19mm zone against *S. aureus*, simultaneously it shows 20mm, 19mm, and 18mm zone of inhibition against *E.coli*, *Salmonella*, and *Vibrio* respectively. It does not showed zone of inhibition against *Bacillus subtilis*, *Pseudomonas* and *A.nigar*.

The data showed antimicrobial activity in ethanol extract of *Aconitum ferox wall* plant. The analysis reflects that this extract shows highest 20 mm zone of inhibition among all against *E. coli*.

Present study found that ethanol extract of *Aconitium ferox wall* extract shows the activity against gram positive and gram negative is an indication that the plant can be source of bioactive substances that could be broad spectrum of activity (Fig No.18).

The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be source of very potent antibiotic substances that can be used against drug resistant microorganism prevalent in hospital environment. *E. coli* (20mm zone of inhibition), *S. aureus* (19 mm zone of inhibition), *Bacillus subtilis* (0 mm zone of inhibition), *Salmonella* (18 mm zone of inhibition), *Aspergillus niger* (0 mm zone of inhibition), *Pseudomonas* (0 mm zone of inhibition) and *Vibrio* (17 mm zone of inhibition).

The analysis reflects that this solvent show highest 20 mm of zone of inhibition against *E.coli*,

it indicates that, this extract can be used to controlling enteric infection.

The all over that show that it does not shows zone of inhibition against *B. subtilis, A. niger* and *Pseudomonas*, this three are found to be resist to this extract.

The study shows the presence of different phytochemical with biological activity that can be valuable therapeutic index. The results of phytochemical in present investigation show that plant contains Alkaloids, Saponins, Flavonoids, Phenol, Tannins, Terpenoid and Carbohydrate. Antimicrobial properties of medicinal plants are being increasingly reported from different part of the world. The world health organization estimated that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% world's population. In the present work, the extract obtained from root of *Aconitum ferox wall* plant shows strong activity against the tested bacterial Strains. The Above results show that the activity of extract of root of *Aconitum ferox wall* shows significant antibacterial activities.

Further, more specific studies, in vivo, are recommended to determine the efficiency of this plant extract in the treatment of gram negative bacterial infection.

The ethanol extract of *Aconitum ferox* wall showed highest antimicrobial activity against to gram negative bacteria as compared to gram positive bacteria and data also showed that it don't show antifungal activity.

Above table depicts the findings of various contents of the plant. According to ancient literature, they are useful in vitiated conditions of pitta, ophthalmology, pruritis, cephalgia, stomatopathy, leprosy, ulcers, fever, vomiting, hiccough, insanity and galactorrhoea [27].

Aconitum is one of the most valuable and toxic drugs. The diesterditerpene alkaloidal nature is responsible for its toxicity. But it can be utilized safely after processing. Assessment of Aconitum species needs to be carried out for their safely use [28].

CONCLUSION

The present work has proved that the extract of root *of Aconitum ferox* wall showed the presence of phytochemical constituents. This plant also possessed strong antimicrobial activity.

In the preview of study when ethanol extract of root of *Aconitum ferox* wall are analyzed following conclusion were obtained from phytochemical study that plant contains Alkaloids, Saponins, Flavonoids, Phenol, Tannins, Terpenoidand Carbohydrate.

The qualitative analysis of various extract of root of *Aconitum ferox* wall shows the presence of bioactive compounds. The results are summarized in the tables.

The ethanol extract of *Aconitum ferox* wall showed highest antimicrobial activity against to gram negative bacteria as compared to gram positive bacteria and data also showed that it don't show antifungal activity.

The present study justified the claimed uses of this plant intraditional system of medicine to treat various infectious diseases caused by microbe.

The present result will form on the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Today, antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health- related quality of human life. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective not only because of many of them produce toxic reactions, but also due to drug emergence of drug resistance bacteria. Drugs derived from natural sources play a significant role in prevention treatment of human diseases.

Aconitum ferox wall exhibited significant, antimicrobial activity and helpful as a future medicinal content.

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REFERENCES:

Gough A, Young A. Setting up an early inflammatory arthritis clinic. ClinRheumatol 1992; 6:261-84.

Ahn k. (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs" BBM report 50(3):111-116 doi:10.5483/BBMRep.2017.50.3.221.PMC 5422022. PMID 27998396

Rios MY, Castrejon F. Chemical composition and Antimicrobial activity of the essential oil from Annona cherimola (Annonaceae). Revista de la sociedadQuimica de Mexico 2003; 47:139-42.

Chisholm, Hugh, ed. (1911). "Aconite". Encyclopædia Britannica. ***1*** *(11th ed.).Cambridge University Press. pp. 151–152.*

Shastry J.L.N., Dravyagunavijnana, (2004), ChaukhambhaOrientalia, Varanasi, Ed.1st,vol.2,pp-9.

Rice JW., Veal JM., Fadden RP., Barabasz AF., Partridge JM., Barta TE., Dubois LG., Huang KH., Mabbett SR., Sillinski MA., Steed PM., Hall SE. Small molecule inhibitors of Hsp90 potently affect inflammatory disease pathways and exhibit activity in models of rheumatoid arthritis. Arthritis and rheumatism. 2008; 58: 3765-3775.

"Active plant ingredient Used for Medicinal Purpose" United State department of agriculture. Retrieved 18 February 2017.

Awanish Pandey, Gyan Chand Kr.Morya, H.S. Mishra, R.B.Yadav and K.N. Yadav. "A Review Study on Therapeutic Potential of Vatsanabha." 2017 Int J Ayu Pharm Chem 2017 Vol. 6 Issue 2-272.

Del R, I, Battafarano DF, Arroyo RA, Murphy FT, Fischbach M, Escalante A. Ethnic variation in the clinical manifestations of rheumatoid arthritis: role of HLA-DRB1 alleles. Arthritis Rheum 2003;49(2):200-8.

"Antimicrobial" Merriam-Webster online dictionary. Archived from the original 24 April 2009. Retrieved 2009-05-02.

"Antimicrobial porous media/ Microbicidal technology/ Porex Barrier technology". www.porex.com. Retrieved 201-02-16.

Brandt FJ (Feb 2013)."American general of Gastroenterology Lecture: Intestinal microbiota and the role of fecal microbiota transplant in treatment of C.difficile infection" Am J Gastroenterol 108(2):117-85 doi : 10.1038/ajg.2012.450 PMID 23318479.

Fransworth NR. Ethno pharmacology and future drug development. The North American experience. J Ethnopharmocol 1993.38.145.52.

Jabeen, N., A.S. Shawl, G.H. Dar and P. Sultan, 2006. Callus induction and organogenesis from explants of *Aconitum heterophyllum*medicinal plant. Biotechnol., 5: 287-291.

Wang, Z., J. Wen, J. Xing and Y. He, 2006. Quantitative determination alkaloids in four species of *Aconitum* by HPLC. J. Pharm. Biomed. Anal., 40: 1031-1034.

Pelltier, S.W., R. Aneja and K.W. Gopinath, 1968. The alkaloids of *Aconitum heterophyllum* Wall: Isolation and characterization. Phytochemistry, 7: 625-635.

Murayama, M., T. Mori, H. Bando and T. Amiya, 1991. Studies on the constituents of *Aconitum* species. IX. The pharmacological properties of pyro-type aconitine alkaloids, components of the processed aconite powder Kako-bushi-matsu: Analgesic, anti-inflammatory and acute toxic activities. J. Ethnopharmacol., 35: 159-164.

Oyama, T., T. Isono, Y. Suzuki and Y. Hayakawa, 1994. Antinociceptive effects of Aconiti tuber and its alkaloids. Am. J. Chin. Med., 22: 175-182.

Ameri, A., 1998. The effects of *Aconitum* alkaloids on the central nervous system. Prog. Neurobiol., 56: 211-235.

Taki, M., Y. Omiya, Y. Suzuki, Y. Ikeda and M. Noguchi *et al.*, 1998. Quality and pharmacological investigation of processed Aconit tuber (TJ-3022). Nat. Med., 52: 348-352.

Jiang, Z.H., Y. Xie, H. Zhou, J.R. Wang and Z.Q. Liu *et al.*, 2005. Quantification of *Aconitum* alkaloids in aconite roots by a modified RP-HPLC method. Phytochem. Anal., 16: 415-421.

Bisset, N.G., 1981. Arrow poisons in China. Part II. Aconitum-botany, chemistry and pharmacology. J. Ethnopharmacol., 4: 247-336.

Lim, C.E., J.K. Park and C.W. Park, 1999. Flavonoid variation of the *Aconitum* jaluense complex (Ranunculaceae) in Korea. Plant Syst. Evol., 218: 125-131.

Treutter, D., 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. Plant Biol., 71: 581-591.

Koes, R.E., F. Quattrocchio and J.N.M. Mol, 1994. The flavonoid biosynthetic pathway in plants: Function and evolution. Bioessays, 16: 123-132

Eva Petrikkou, Juan L. Rodriguez-Tudela, and Emilia Mellado.

Babbar N., Casero R. Tumor necrosis factor-alpha increasres reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. Cancer Res. 2006; 66: 11125-11130.

ShyaulaSajan L. Phytochemicals, Traditional Uses and Processing of Aconitum Species in Nepal. Nepal Journal of Science and Technology 2011; 12: 171-178.

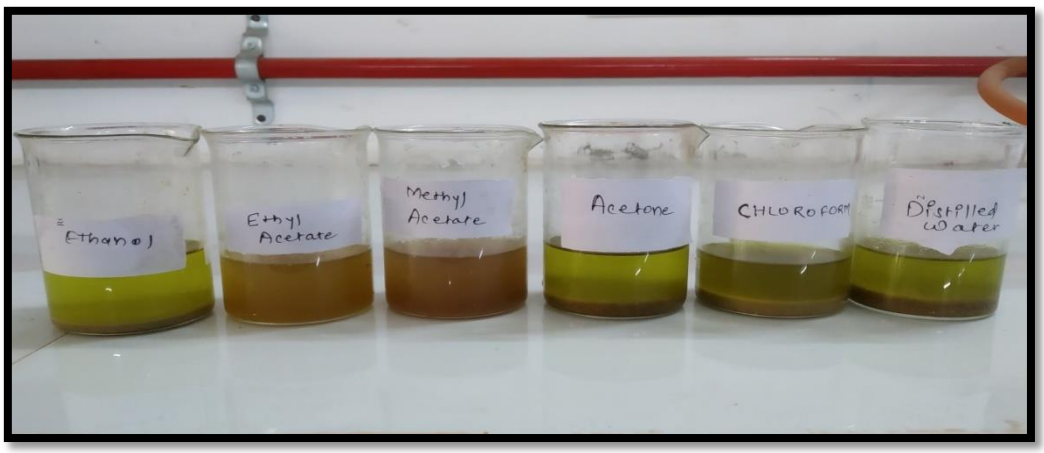

Fig No. 3:- Extraction of *Aconitum ferox wall* in different Solvents.

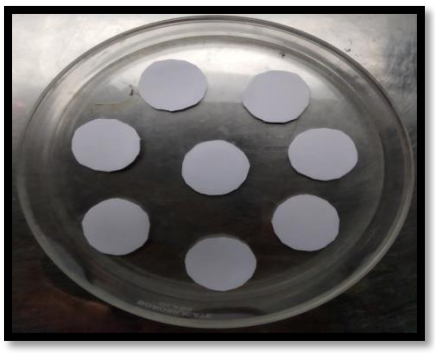	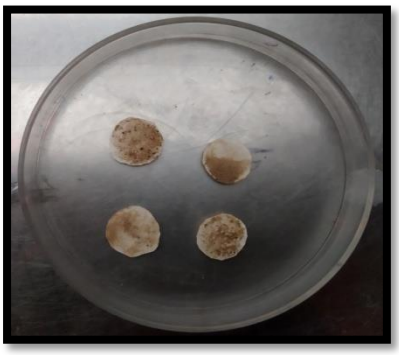
Fig No. 5 Sterile Disc	**Fig No. 4: Soaked Disc.**

Table No.1 Bacteria Used for Antibacterial activity

Sr. No.	Organisms
1.	***S. aureus***
2.	***E.coli***
3.	***Salmonella***
4.	***Bacillus Subtilis***
5.	***Vibrio***
6.	***Pseudomonas***

Sr. No.	Fungi
1.	***Aspergillus niger***

Medium Used:-

Table No. 2:- Mueller-Hinton medium

Sr. No.	Content	Concentration (g/L)
1.	Beef infusion	300.0 g
2.	Casamino acids	17.5 g
3.	Starch	1.5 g
4.	Agar	17.0 g
5.	Distilled Water	1000.0 ml

Table No. 3:- Nutrient Agar Medium

Sr.No.	Content	Concentration(g/L)
1.	Peptone	5.0 g
2.	Beef Extract	3.0 g
3.	NaCl	5.0 g
4.	Distilled Water	1000.0 g
5.	Agar-Agar	15.0 g

Table No. 4:- Potato Dextrose Agar (PDA) Medium

Sr.No.	Content	Concentration (g/L)
1.	Potato infusion	200 gm
2.	Dextrose	20 gm
3.	Agar-Agar	20 gm
4.	Distilled Water	1000 ml

Table No. 5:- Sabouraud Dextrose Agar (SDA) Medium

Sr. No.	Content	Concentration (g/L)
1.	Dextrose (glucose)	40 gm
2.	Peptone	10 gm
3.	Agar-Agar	15 gm
4.	Distilled Water	1000 ml

Table No. 6:- Nutrient Broth

Sr. No.	Content	Concentration(g/L)
1.	Beef Extract	1 gm
2.	Yeast Extract	2 gm
3.	Peptone	5 gm
4.	Sodium Chloride	5 gm
5.	Distilled Water	1000.00 ml

Table No. 7:- Mullen-Hinton Agar, 2% Glucose with Methylene Blue

Sr. No.	Content	Concentration (g/L)
1.	Beef Infusion Form	300.00
2.	Casein Acid Hydrolysate	17.500
3.	Starch	1.500
4.	Glucose	20.000
5.	Methylene Blue	0.0005
6.	Agar	17.00
7.	P^H (at 25°C)	7.3 ± 0.1

Table No.8:-Phytochemical activity of root of extract of *Aconitum ferox wall*

Sr.No.	Test/ Reagents Used	Ethanol Extract	Chloroform Extract	Ethyl Acetate Extract	Methyl Acetate Extract	Acetone	Distilled Water
1	Alkaloids(wagner's test)	+	+	+	+	-	+
2	Glycosides(Liebermann'sTest)	-	-	-	-	-	-
3	Phenols	+	-	-	+	-	+
4	Saponins(Foam test)	+	+	+	-	-	-
5	Tannins(Braymer's test)	-	-	-	-	-	-
6	Flavonoids (Shinoda's test)	+	-	-	-	+	+
7	Terpenoids	+	-	-	+	-	-
8	Phlobatanins	-	-	-	-	-	-
9	Anthraquinones	-	-	-	-	-	-
10	Carbohydrates(Molisch'stest)	+	-	-	+	-	-

Table No. 9:- Antimicrobial Activity (mm)

Sr. No.	Microorganisms	Zone of inhibition
1.	*S. aureus*	19 mm
2.	*Bacillus subtilis*	00 mm
3.	*E.coli*	20 mm
4.	*Salmonella*	18 mm
5.	*Pseudomonas*	00 mm
6.	*Vibrio*	17 mm

Sr. No.	Fungi	Zone of inhibition
1.	*A. niger*	00 mm