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Invitro Antibacterial Activity of Centellaasiatica and Azadirachta indica against Pasteurella multocida Isolated from Goat

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ABSTRACT: Ovine respiratory diseases like shipping fever, pneumonia, sinusitis and rhinitis have become prevalent these days due do various factors like nasal bots, ammonia vapours, dusty feeds, improper sanitation and pathogenic microbes like Pasteurella multocida, Manheimahaemolytica and Mycoplasma ovipneumoniae. These factors often trigger the organism to act against the immune system and make it pathogenic causing severe discomfort to the animal. Most probably the clinical signs associated with these diseases include nasal discharges. Pasteurella multocida infections in ovine species often result in nasal discharges that act as a carrier for disease transmission from goat to humans. The present study was carried out by using two plant sources such as Centellaasiatica and Azadirachtaindica to check the antibacterial effect of the plants against Pasteurellamultocida that was isolated from nasal swab of a two-year-old male goat. The organism was isolated and confirmed by 16s r RNA gene sequencing. Ethanol, acetone, benzene, chloroform and water were used as the solvents. The acetone and chloroform extracts showed high range of inhibition activity compared to aqueous, ethanol and benzene extracts. Also, the presence of various phytochemicals like tannins, flavonoids, cardiac glycosides, alkaloids, saponins, reducing sugar phenol, terpenoids, volatile oils and amino acids were confirmed. This study showed that the organism was susceptible to both the plants since they contained healthy natural phytochemicals and the plants could be used as a regular food supplement to cure nasal discharges in goat.

KEYWORDS: Pasteurellamultocida,Centellaasiatica,Azadirachtaindica**,** nasal discharge, antibacterial activity.

I. INTRODUCTION

Ovine respiratory diseases have become prevalent these days due to improper sanitation and polluted environment which acts as a channel for transmittance of pathogenic and non-pathogenic microbes. Bacterial respiratory diseases include, primary acute to bronchopneumonia, which is caused by secondary pathogens like Pasteurella multocida, Manheimahaemolytica, Mycoplasma ovipneumoniae, and Chlamydia pneumoniae (Gonzalez, 2015). Clinical signs associated with such infections in ovine species include, bilateral to unilateral mucopurulent or serous nasal discharges, fever or hyperpnoea, lack of airflow in either of the nostrils, coughing, sneezing and mild to severe respiratory distress could also be seen (The Merck Veterinary Manual). Unilateral nasal discharges are localized conditions that involve the nose or sinus regions of the upper respiratory tract whereas bilateral nasal discharges indicate regions involving the lower respiratory tract especially the thoracic cavity (Veterinary medicine, 10th edition 2007). Pasteurella multocida being a common commensal in most domesticated animals like sheep, goat, dog, cat, rabbit and cows become virulent due to factors like stress, fever, poor hygiene, improper intake of food, and nasal bots (Gonzalez et al., 2016). These factors often trigger the organism to act against the immune system and make it pathogenic causing severe discomfort to the animal (Bello et al., 2019). Pasteurella multocida infections in ovine species often result in nasal discharges that act as a carrier for disease transmission from goat to humans. When the organism gains entry into the host, via handling methods, bites or direct pet handling, transportation etc., (Brogden et al., 1998) it causes infection in the soft tissues of skin which may lead to erythema and pain in the upper layer of skin. It may also promote tenderness and swelling. Also, lymphangitis and localized lymphadenopathy can be seen. Other complications include tenosynovitis and formation of abscess in the soft tissue (David J Weber et al., 1984). The second most prevalent

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source of an infection with Pasteurella concerns the respiratory tract. Tracheobronchitis, otitis, sinusitis, mastoiditis and pneumonia with pus in the pleural cavity have been reported. The majority of patients having lower respiratory tract infections with Pasteurella are older and have problems like bronchiectasis and chronic obstructive pulmonary disease. The signs of these infections are most probably not marked by eminence from various other cause of respiratory lining infections. Pneumonia is most usually focal in appearance but in some patients, it has been identified as diffuse (Klein and Cunha, 1997). It is known that pneumonic Pasteurellosis is highly responsible for causing death in most of the domesticated feedlot animals (Mohammed and Abdelsalam, 2008).Once the goat is affected the disease spreads easily to other livestock's and cause economic loss to the concerned industry (Gu CJ et al., 2013). Many antibiotic susceptibility studies are done to test the resistance or sensitivity of the organism to a wide range of antibiotics. Nasal discharges in goats are not often treated. They are left as such because the infection is not severe. They are taken for treatment only when the symptoms are severe. Antibiotics like tetracycline, penicillin, ampicillin, carbenicillin, cephalothin and chloramphenicol are active drugs against this pathogen (Stevens et al., 1979). Vaccines that are effective have been developed. This includes the alum precipitated and oil adjuvant vaccine (Chandrasekaran et al., 1994; Myint and Jones, 2007).This study is to provide natural herbs that could replace chemotherapeutic agents to act against Pasteurella multocida.In this study two herbal plants, Centellaasiatica (Asian Pennywort) and Azadirachtaindica (Neem) are used. The chemical composition of Centellaasiatica includes pentacyclic triterpenoids as well as asiaticoside, brahmoside, asiatic acid and brahmic acid (madecassic acid). Centellose, centelloside and madecassoside are various other constituents of this plant (Singh et al., 1969; Singh et al., 1968; Murray, Textbook of natural medicine). Triterpenoid and saponins the main constituents of Centellaasiatica are thought to be mainly responsible for their wide-ranging therapeutic behaviour (Gohil et al., 2010). This plant has attributed various health benefits like preventing Alzheimer's disease, blood clots and infections like common cold, swine flu, cholera and shingles. Apart from these it could also treat or prevent anxiety, depression, asthma, diabetes, diarrhoea, tiredness, digestion problems and ulcers in the stomach (Brinkhaus et al., 2000). Another great potential of this plant is that it can act against some bacterial species like Pseudomonas pyocyaneus and Trichoderma mentagrophytes (Tschesche and Wulff, 1965) and also viruses like Type 2 Herpes simplex virus (HSV-2) (Zheng, 1989). An important characteristic feature of this herb is that it possesses anti-catarrhal property which eliminates or prevent mucus formation (Kartnig, 1988).Neem is a significant source of traditional medicinal products in the Ayurvedic, Unani, Tibbi and various tribal medicinal systems (Benthall, 1984; Dastur, 1962; Jain, 1991). Several sections of the tree are recorded to have analgesic, antiperiodic, antiseptic, anthelmintic, antipyretic, diuretic and anti-syphilitic properties. It can also be used to relieve irritation or inflammation. It is also used as an emmenagogue, astringent, emollient and a purgative. Medicinal products made from the compounds of neem tree were used to treat diseases in eyes. Also, it can be provided to cure boils, eczema, headaches, hepatitis, malaria, scrofula, ulcers, rheumatism and leprosy (Chopra et al., 1956; Dastur, 1962; Ketkar, 1976; NRC, 1992).It also acts as a killing agent against various pathogenic microbes like bacteria, fungi and virus. It also possesses anti diabetic property (NRC, 1992). The margosa oil or the acrid oil that is deep yellow in colour extracted from the seeds of this tree has been used in the conventional Indian medicine for its anthelmintic and antiseptic nature and as an alternative method for treating rheumatism (NRC, 1992). A compound, 9-octadecanoic acid-hexadecenoic acid-tetrahydrofuran-3,4-diyl ester from the neem oil has been proven to possess the antibacterial activity (Zhong et al., 2010).

II. MATERIALS AND METHODS

2.1 Plant collection

The leaves of the plants, Centellaasiatica (Asian pennywort)and Azadirachtaindica (Neem) used in this studywere collected from the village of Kozhikarai, Clovely Estate, The Nilgiris district, TamilNadu, India.

2.2Processing of plants

The collected plants were washed carefully 2-3 times in sterilized water to remove excess dirt and mud. Using clean tissue papers, excess water was drained out. Leaves of both the plants were dried separately under shade for about three weeks and was then finely powdered using a mixture grinder. The powdered leaves were stored

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in suitable containers for further analysis (Maragathavalli et al., 2012).

2.3 Preparation of crude extract

The powdered leaves were infused in various polar and non-polar solvents in order to get the crude extract. 2.5 grams of powdered Centellaasiatica and Azadirachtaindicawas mixed with 25 millilitres of the organic solvents (acetone, ethanol, benzene, chloroform) and the inorganic solvent (distilled water) separately in two different conical flasks, sealed with aluminium foil and placed in a rotatory shaker for about 72 hours. After 72 hours the solvent mixture was filtered primarily in muslin cloth and re-filtered using Whatman No.1 filter paper (Jyothi Chaitanya et al., 2013). The filtered extract was transferred to sterile petri dishes for evaporation . The crude extract obtained is stored at 4℃.

2.4 Collection of clinical specimens:

Bacterial strain used in this study is the pathogen isolated from the nasal swab of goat. The mucopurulent nasal discharge was collected using a sterile swab from a male goat that was two years old. It was collected from Government Veterinary Hospital, Vedapatty, Coimbatore. The nose region of the goat was first cleaned with spirit and the yellowish discharge was collected (Momin et al., 2011). The collected specimen was transported immediately to laboratory under cold condition.

2.5 Processing of clinical specimen

The collected clinical specimen was processed by suspending the nasal swabs in 0.5 ml Phosphate buffer saline (Nidhi Rawat et al., 2019). Further swabbing of the nasal mucus was done on generalized as well as specific agar media to isolate individual colonies. Nutrient agar, Blood agar, MacConkey agar, Heart infusion agar and Mueller Hinton agar were used for the isolation of individual colonies. All the processing techniques were performed under sterile aseptic conditions using a Laminar Air Flow Chamber.

2.6 Isolation of Pasteurella multocida

Initially the collected sample was directly inoculated on Nutrient Agar plates under aseptic conditions by swabbing the nasal mucus on the surface of the agar media . The plate was incubated at 37℃ for 24 hours. The Nutrient Agar plate was checked for colony growth the following day. Suspected colony was selected and streaked on Blood Agar plates using Quadrant streak method . The plates were incubated at 37℃ for 24 hours. Colony morphology by Gram staining and colour of the pure culture was examined (Nidhi Rawat et al., 2019).Using a sterilized nichrome microstreaker, selected individual colonies were picked and subcultured on MacConkey Agar and Brain Heart Infusion Agar and incubated at 37℃ for 24 hours. The pure isolate of the selected colony was inoculated in Brain Heart Infusion Broth and incubated at 37℃ for 24 hours. The stock culture was used for further testing processes (Nidhi Rawat et al., 2019).

2.7 Identification of Pasteurella multocida

Identification and Characterization of the isolated colony was done according to standard protocols. Grams staining was done to examine the morphology of the bacteria. The isolates were subjected to various tests to find out the following characteristics such as, growth of the organism on MacConkey Agar, haemolysis or non-haemolysis in Blood Agar, production of indole, fermentation of sugars etc. The biochemical tests like IMViC test, Catalase test, Oxidase test, Carbohydrate fermentation test, Triple Sugar Iron test, Hydrogen sulphide production test and Gelatin liquefaction test were performed (Quinn et al., 2001).

2.8 Molecular Identification of the isolated organism by 16s rRNA Gene Sequencing

Molecular identification of the isolated organism by 16s rRNA sequencing using universal primers is done.

2.9 Antibacterial Activity of Centellaasiatica and Azadirachtaindica

The dried plant extract was mixed with each solvents Acetone, Benzene, Chloroform, and Ethanol along with Dimethyl-Formamide in the ratio 1:1. Agar disc diffusion and agar well diffusion method was performed.

2.9.1 Disc diffusion method (P.Jyothi Chaitanya et al., 2013)

Mueller Hinton Agar was prepared and sterilized. The sterilized media was poured in sterile petri dishes and kept for solidification of the media. After solidification, previously inoculated culture from heart infusion broth was taken and swabbed in the plates. The plate was allowed to dry for few minutes . Whatman filter paper was cut as discs and sterilized at 121℃ for 15-20 minutes under 15 lbs pressure per square inch. 10 micro litres of each solvents containing plant extract was

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taken using a micropipette and placed in each disc and is allowed to dry. The discs were impregnated on the agar surface using sterile forceps placed few centimetres apart. Gentamycin and Azithromycin were used as a standard control. The plates were kept for incubation at 37℃ for 24 hours. Following incubation, the plates were examined for zone formation.

2.9.2 Well diffusion method: (P.Jyothi Chaitanya et al., 2013)

Mueller Hinton Agar was prepared and sterilized. The sterilized media was poured in sterile petri dishes and kept for solidification of the media. After solidification, previously inoculated culture from heart infusion broth was taken and swabbed in the plates. The plate was allowed to dry for few minutes. Wells were cut on the agar plates using a cup borer at a depth of 4 mm. To the wells, respective solvents containing plant extract were added. Meropenem and Ciprofloxacin were used as a standard control . The plates were kept for incubation at 37℃ for 24 hours. Following incubation, the plates were examined for zone formation.

2.10 Phytochemical Analysis of Centellaasiatica and Azadirachtaindica

Serious of tests were done to detect the presence of alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, volatile oils, phenols, reducing sugars, amino acids and steroids.

Detection of alkaloids (Chanda et al., 2006)

Wagner's test: 2 ml of the filtrate was added to 1% Hydrochloric acid and steam was passed. To this solution 6 drops of Wagner's reagent was added. Brownish red precipitate will indicate the presence of alkaloids.

Detection of cardiac glycosides (Parekh and Chanda, 2007)

Kellar - Killiani test: To 2 ml of the filtrate, 1 ml of glacial acetic acid, ferric chloride and concentrated sulphuric acid were added. Green blue colouration of the solution will indicate the presence of cardiac glycosides.

III. RESULT

3.1 Isolation of Pasteurella multocida

The isolated colonies produced the following results.

Nutrient agar medium: Primary culturing of nasal swab in nutrient agar medium. Small, whitish, opaque, circular and translucent single colony was observed.

Blood agar medium: Non- haemolytic circular, slight grey, opaque colonies were observed.

MacConkey medium: No growth of colonies was observed in MacConkey plates.

Detection of flavonoids (Kumar et al., 2007)

Shinoda test: A piece of magnesium ribbon and 1 milli liter of concentrated hydrochloric acid was added to 2 to 3 ml of the extract. Pink red or red colour of the mixture will indicate the presence of flavonoids.

Detection of phenol (Kumar et al., 2007)

Phenol test: A spot of the extract was placed in a filter paper. To this spot, 1 drop of phosphomolybdic acid was added and exposed to vapours of ammonia. Blue colouration of the spot will indicate the presence of phenols.

Detection of saponin (Parekh and Chanda, 2007)

Frothing test/ Foam test: To 0.5 ml of the filtrate, 5 ml of distilled water was added and vigorously agitated. Persistent froth will indicate the presence of saponins.

Detection of steroid (Edeoga et al., 2005)

2 ml of concentrated sulphuric acid and acetic anhydride was added to 1 ml of the filtrate. Colour change to blue or green will indicate the presence of steroid.

Detection of terpenoid (Edeoga et al., 2005)

Salkowski test: To 5ml of the extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added. Reddish brown colour of the interface will indicate the presence of terpenoids.

Detection of amino acids (Chandrashekar et al., 2013)

Few drops of the ninhydrin reagent was added to 1 ml of the extract. Formation of purple colour will indicate the presence of amino acids.

Detection of reducing sugars (Akinyemi et al., 2005)

Fehling's test: To 1 ml of the filtrate, 5 to 10 drops of Fehling's solution was added and boiled for 15 minutes. Formation of brick red precipitate will indicate the presence of reducing sugars.

Detection of volatile oil (Dahiru et al., 2006)

0.1 ml dilute NaOH and few drops of dilute HCl were added to 2 ml of the extract. The mixture was shaken well. Appearance of white precipitate will indicate the presence of volatile oils.

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Gram staining: Short rods or coccoid rods in pairs, single or as short chains were observed. It was similar to coccobacilli. The organism stained pink which indicates that the organism is Gram Negative.

Table 1: Culture characteristics

Medium/Procedure	Characteristics
Blood agar	Small mucoid grey non- haemolytic colonies.
Nutrient agar	White, circular, opaque colonies
Mac Conkey agar	No growth
Gram staining	Gram negative

Table 2: Biochemical tests

Test	Result
Catalase	Positive
Oxidase	Positive
H2s test	Negative
Indole	Positive
Methyl red	Negative
Voges Proskauer	Negative
Citrate	Negative
Carbohydrate fermentation-Glucose	Positive
Carbohydrate fermentation-Lactose	Negative
Carbohydrate fermentation-Gas production	Negative
Gelatin liquefaction	negative
TSI test	Acid slant/ Alkaline butt

3.2 Molecular characterization of the organism

Genetic code: > Contig - KO9

CGGGAAACCGAAGCTAATACCGGA
TAGGATCTTCTCCTTCATGGGAGATGATTG
AAAGATGGTTTCGGCTATCACTTACAGATG
GGCCCGCGGTGCATTAGCTAGTTGGTGAGG
TAACGGCTCACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCGCAATGGACG
AAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTAGGGAAGAACAAGTACGAGAGTAAC
TGCTCGTACCTTGACGGTACCTAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGTGGCAAGCGTTATCCGG
AATTATTGGGCGTAAAGCGCGCGCAGGCGG
TTTCTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGGG
AACTTGAGTGCAGAAGAGAAAAGCGGAAT
TCCACGTGTAGCGGTGAAATGCGTAGAGAT
GTGGAGGAACACCAGTGGCGAAGGCGGCT
TTTTGGTCTGTAACTGACGCTGAGGCGCGA
AAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGAGTG
CTAAGTGTTAGAGGGTTTCCGCCCTTTAGT
GCTGCAGCTAACGCATTAAGCACTCCGCCT
GGGGAGTACGGTCGCAAGACTGAAACTCA
AAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAGGTCTTGACATCCTCT
GACAACTCTAGAGATAGAGCGTTCCCCTTC
CGGGGACAGAGTGACAGGTGGTG

RESULT: **Pasteurella multocida**

3.2.1 Blast results

Graphic Summary

Distribution of the top 200 Blast Hits on 100 subject sequences

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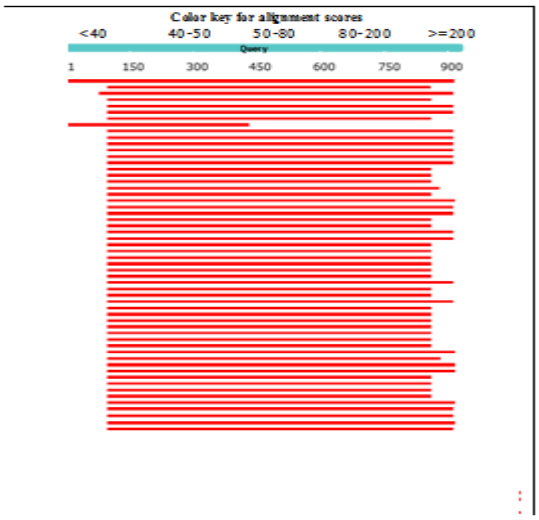

Phylogenetic tree showing Pasteurella multocida K09

3.3 Phyto chemical analysis

The phytochemical analysis of both the plants showed the presence of various compounds like alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, terpenoids, amino acids, reducing sugars and volatile oils.

Table 3: Acetone extract of Centellaasiatica and Chloroform extract of Azadirachtaindica showed the presence and absence of various phytoconstituents present in the plant.

S. No	Phytochemicals	Centellaasiatica (acetone extract)	Azadirachtaindica (chloroform extract)
1	Alkaloids	+	-
2	Cardiac glycosides	-	+
3	Flavonoids	+	-
4	Phenols	+	+
5	Saponins	+	+
6	Steroids	-	+
7	Terpenoids	+	+
8	Amino acids	+	+
9	Reducing sugars	+	-
10	Volatile oils	+	+

(+ indicates positive result and – indicates negative result)

3.4 Anti-bacterial activity

The antibacterial activity of Centellaasiatica and Azadirachtaindicawas performedagainst the organism Pasteurella multocida that has been isolated. Agar disc diffusion and well diffusion method performed showed the following results.

3.4.1 Agar disc diffusion method

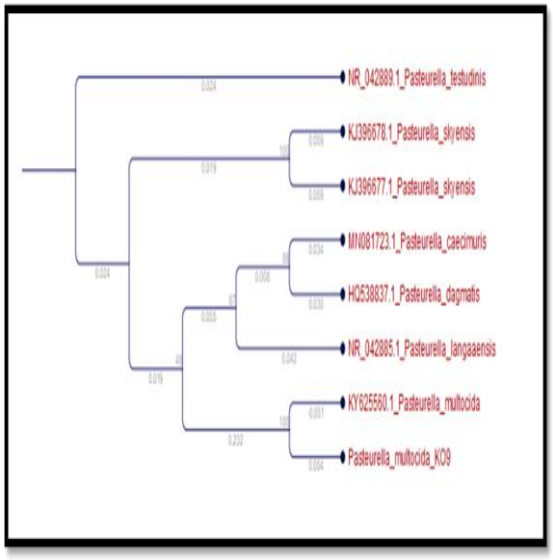

Fig- 1: Aqueous extract of Centellaasiatica and Azadirachtaindica showed no zone of inhibition.

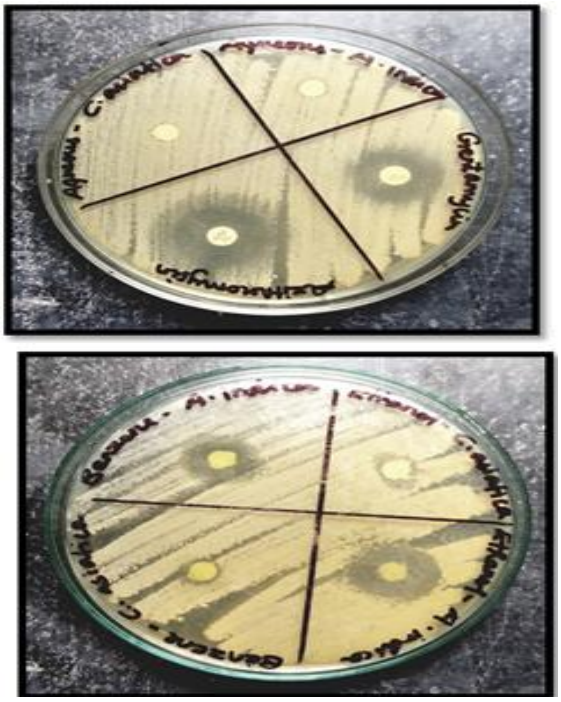

Fig- 2: Ethanol and benzene extracts of Centellaasiatica and Azadirachtaindica showed characteristic zones of inhibition

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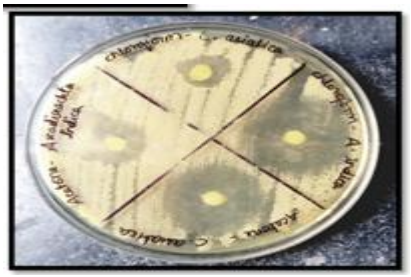

Fig- 3: Acetone and chloroform extract of Centellaasiatica and Azadirachtaindicashowed maximum zone of inhibition.

Table 4: Antibacterial activity of the plant's inhibition zone by disc measured in Diameter

Solvent	Zone of inhibition (mm) of Centellaasiatica	Zone of inhibition (mm) of Azadirachtaindica	Gentamycin (mm)	Azithromycin (mm)
Acetone	25	16	19	22
Benzene	6	15		
Aqueous	No zone	No zone		
Chloroform	15	26		
Ethanol	11	16		

3.4.2 Agar well diffusion method

Fig-4: Benzene and ethanol extracts of Centellaasiatica and Azadirachtaindica showed characteristic zones of inhibition.

Fig-5: Aqueous extract of Centellaasiatica and Azadirachtaindica showed characteristic zone of inhibition compared to disc diffusion technique.

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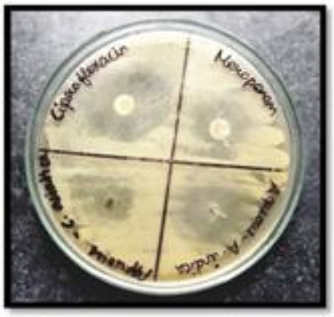

Fig-6: Acetone extract of Centellaasiatica and chloroform extract of Azadirachtaindica showed maximum zone of inhibition.

Table 5: Antibacterial activity of the plant's inhibition zone by well measured in Diameter

Solvent	Zone of inhibition (mm) of Centellaasiatica	Zone of inhibition (mm) of Azadirachtaindica	Meropenem (mm)	Ciprofloxacin (mm)
Acetone	20	15	13	25
Benzene	15	15		
Aqueous	10	12		
Chloroform	12	18		
Ethanol	14	17		

IV. DISCUSSION

The use of natural plants to cure a wide variety of diseases has been predominantly researched. In this study two herbal plants Centellaasiatica and Azadirachtaindicathat were used showed its medicinal property in inhibiting the growth of the pathogen, Pasteurella multocidathat was isolated from nasal swab of goat.The organism was confirmed by various biochemical tests. The gram negative coccobacillary organism showed no growth on MacConkey agar and was positive for indole test. The organism fermented only glucose and not lactose which are the characteristic features of this organism. The results were similar to the findings done by Belal, (2013).The phytochemical screening of the plant Centellaasiatica showed the presence of alkaloids, flavanoids, saponins, terpenoids, amino acids, reducing sugars, phenols and volatile oils (Saranya et al., 2017) that were responsible for inhibiting the growth of the pathogen.The phytochemical screening of the plant Azadirachtaindica showed the presence of saponins, terpenoids, cardiac glycosides, amino acids, volatile oils, phenols and terpenoids (Maragathavalli et al., 2012) that were responsible for inhibiting the growth of the pathogen. From the study of (Saranya et al., 2017) Centellaasiaticais known to possess various phytoconstituents like flavanoids, saponins, alkaloids and terpenoids that are used as an antimicrobial and antidiarrheal agent. Also, from the study of (Maragathavalli et al., 2012) the compounds like saponins, glycosides and flavanoids possess antibacterial efficacy. The polar solvents, acetone and ethanol gave wide range of inhibition spectrum compared to the non-polar solvents, benzene and chloroform but no zone of inhibition was seen in the aqueous extract in the disc diffusion technique. This may be due to the less diffusion of the disc since it acted as a barrier between the solvent and the organism. Acetone extract of Centellaasiatia (25 mm) and chloroform extract of Azadirachtaindica (26 mm) showed the maximum range of inhibition. In the well diffusion technique, both the polar and non-polar solvents showed characteristic inhibition against the pathogen. Acetone (20 mm) and benzene (15 mm) extract of Centellaasiatica and chloroform (18 mm) and ethanol (17 mm) extract of Azadirachtaindica showed maximum zone of inhibition. The antibiotics such as gentamycin, azithromycin, meropenem and ciprofloxacin at 10 mg concentration were used as standard positive controls. Ciprofloxacin gave maximum zone of inhibition of 25 mm and Meropenem gave the minimum inhibition zone of 13 mm.

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V. CONCLUSION

The present study implies that natural plants like Centellaasiatica and Azadirachtaindica could be used as a curative drug to cease the nasal discharges caused by Pasteurella multocida. Since the organism is sensitive to the plant's phytochemical activities, the growth of the microbe is controlled and thus these plants could be used in formulating herbal drugs for the treatment of nasal discharges. Extraction of the phytochemicals by using both polar and non-polar solvents of both the plants provided a significant approach to analyse the inhibitory level against the isolated organism. The range of inhibitory activity shown by both plants are equal. Thus, either of the plants can be given as a food supplement in regular diet of the goat that helps in providing immunity without any adverse side effects normally induced by artificial chemical drugs.

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