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THERAPEUTIC INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF *WITHANIA SOMNIFERA, TERMINALIA ARJUNA, BACOPA MONNIERI, RANUNCULUS SCELERATUS* AND *ACALYPHA INDICA.*

Debanjana Prasad[1], Shailesh Solanki[2]

[1] Ph.D. Scholar, Department Of Biotechnology And Microbiology, Noida International University, Greater Noida, U.P.

[2] Associate Professor, Department Of Agriculture And Environment, Noida International University, Greater Noida, U.P.

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Abstract

The present work was carried out using root parts of five medicinal plants from interior areas of Agrakhal of Uttrakhand and surajpur of Uttar Pradesh, India to collect the information of these medicinal plants used traditionally. The objective of this work was to investigate and compare, phytochemical analysis, qualitatively, antimicrobial screening activity of root extractsof *Withania somnifera, Terminalia arjuna, Bacopa monnieri, Ranunculus sceleratus* and*Acalypha indica*by using solvents Ethanol, Methanol and Chloroform**.** In Qualitative Analysis, major active compounds were determined. Antimicrobial screening including Minimum Inhibitory Concentration was evaluated by Disc Diffusion method with four standard antibacterial agents Ciprofloxacin, Tobramycin, Streptomycin, Azithromycin and two standard antifungal agents Fluconazole, Itraconazole. Qualiitative Screening results showed, high presence of beneficial active compounds including alkaloids, coumarin, flavonoids, phenols, tannins in ethanol and methanol extracts. Disc Diffusion Assay revealed maximum potential antimicrobial activity at concentration 10mg/ml against microbes *Bacillus subtilis*(MTCC 441)*:*18mm-24.9mm, *Staphylococcus aureus*(MTCC 96):17mm-19.9mm, *Pseudomonas aeruginosa* (MTCC 647):16mm-18.3mm,

Acinetobactorbaumanni(MTCC 1425):17.1mm-27.1mm, *Saccharomyces cerevisiae*(MTCC 250):15.9mm-22.2mmand *Aspergillus niger*(MTCC 9652):14.8mm-20.3mm were exhibited by these extracts. The MIC was carried out at different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.0625mg/ml). The MIC values of ethanolic and methanolic root extracts were noted to have better function at low MIC values started at 0.625mg/ml against bacteria and 1.25mg/ml against fungi and yeast, respectively as compared with standard agents. It was reported, these ethanol-methanol root extractsare highly effective the for high potential medicinal properties.

Keywords: Medicinal Plants, Phytochemicals,Antimicrobial, Minimum Inhibitory Concentration, Root Extract.

Introduction

The Traditional use of herbal medicine along with herbal medicinal products in developing as well as developed countries, are elevating day by day because of their originality with very less side effects.Due to the structural complexity, lack of toxic effects and antimicrobial broad spectrum the herbal medicines are called to be therapeutic candidates (Mukhtar et al., 2008).Multiple drug resistance function by notorius pathogens has increased care of researchers for searching natural compounds sources with high antimicrobial potential (Ashraf et al., 2015; Nita et al., 2002) Bioactive medicinal compounds extraction shows the physiological activity and facilitates highly potent drug with no toxicity (Sakagami et al., 2001).

Agrakhal in Uttarakhand of the Himalayas is one of the majestic natural beauty having great wealth of medicinal plants along with traditional medicinal knowledge. On the other hand, Surajpur area in Uttar Pradesh, containing reserve forests and wetlands, consists of various diverse range of biodiversity hotspots of flora and fauna, representing the huge collection of habitats and providing medicinal plant species uniqueness. No antimicrobial studies has been yet reported from these areas on medicinal plants including *Withania somnifera, Terminalia arjuna, Bacopa monnieri, Ranunculus sceleratus* and *Acalypha indica*.Ancient Indianscriptureshas reported abundant medicinal values of plants from these areas (Sharma et al., 2011; Naini and Mamidala, 2013). It contains huge source of alkaloids, flavonoids, phenols,chalcones, coumarines, polyketides, alkanes, alkynes, simple aromatics, terpenenoidsetc with high therapeutic potential (Panchangam et al., 2016; Jee et al., 2016). Plants are

mainly used in potent drug discovery in the current era, due its enormous medicinal properties,(Gordon and Newman, 2013; Sen and Chakraborty, 2017).Phytomedicines are an important role in drug discovery system, the health management systems in India and other countries.(Mumtaz et al., 2017). After many decades, recently medicinal plants have been given a wide attention with global recognition in biotechnology area internationally (Pathania et al., 2015; Thakur et al., 2015).

Withania somnifera- It is commonly known as Ashwagandha in hindi, Indian ginseng, solanaceae. The most beneficial and valuable herb, a woody shrub used in traditional Indian medicine (Ulka and Karadge, 2010; Ateb and Erdo, 2003). It originates from India, Mediterranean, Sri Lanka and Africa. Roots are tremendously therapeutic (Al-Hindawi et al., 1992; Bhattacharya et al., 1997). Phytochemical constituents includes fatty acids, organic acids, amino acids, sugars, flavones, and sterol derivatives alkaloids, tannins, flavonoids, terpenes, including choline, tropanol, novel biochemical molecules-withaferin, withanolides. But mainly Withaferin A, withanolide D, *Withania somnifera* glycoprotein possess as a major biologically active constituent (Singh et al., 2010). Acts as antioxidant, antimicrobial, dietary supplement, thickening milk nutritive value (Facciola, 1990; Schliebs et al., 1997; Kulkarni and Ninan, 1997). Prevents parkinson's disease, cures attention deficit hyperactivity disorder (ADHD), Alzheimer's disorder and cerebral ischemia (Nagashayana et al., 2000; Katz et al., 2010) wounds, cough, asthma, diabetes, tumors, hemiplegia, dyspepsia, diarrhoea, etc (Bone, 1996; Grierson and Afolayan, 1999; Acharyya et al., 2009). It has anti-carcinogenic effects reduces tumor size (Prakash et al., 2002; Girdhari and Rana, 2007;Jayaprakasam et al., 2013).

Terminalia arjuna-Commonly as Arjun as well in hindi. A deciduous-evergreen long tree distributed all over India, Burma, Sri Lanka and Mauritius (Ahmed et al., 1983). It consists of tannins which includes Pyrocatechols, Punicallin, Castalagin, Casuariin, Punicalagin, Terchebulin, Terflavin C, steroids, minerals such as calcium, magnesium, zinc, copper, amino acids, carbohydrates, alkaloids, flavonoids like arjunolone, flavones, bicalein, quercetin, kempferol and pelorgonidin (Dhar et al., 1968; Dwivedi, 2007). Triterpenoids mailny arjunin, arjunetin, arjunic acid, arjugenin, glycosides namely Termiarjunoside 1,

Termiarjunoside 2 and Arjunoglycoside, Triterpane, terminoside A, phenols showing antimicrobial properties (Ahmed et al., 1983;Cowan, 1999; Sher, 2009; Das et al., 2010). Cures obesity, hypertension has higher antioxidant potentialcontaining higher amount of phenolic and flavonoids (Amalraj and Gopi, 2017; Singh et al., 2014). Mainly use for having healthy cardiovascular system as heart tonic (Aneja et al., 2012). Acts as anticancer, antiviral and antimicrobial activities (Tripathi et al., 1992; Dwivedi, 2007) anti-dysentric, antipyretic, astringent, cardiotonic, lithotriptic, antimicrobial, antiuremic cardioprotective, anti-inflammatory, immunomodulatory and antinociceptive activity (Modak, 2007), myocardial infarction, degenerative neurological diseases etc.

Bacopa monnieri-Bacopa monnieri known as Brahmi in hindi and commonly as water hyssop. A perennial herb from family Schrophulariaceae. It exits in wet-warm areas. Native to Australia, United States, East Asia, Nepal, Sri Lanka (Barrett and Strother, 1978). It is mainly useful for brain disorders, but also utilise for insanity, epilepsy, skin disease, tumours etc (Ramawat, 2004). Bioactive compounds are alkaloids like nicotine, brahmin, herpestine, saponins including hersaponin, betulic acid and bacosides, chemicals like stigmastanol, saponins A, B and C, triterpenoid saponins, stigma-sterol, flavonoids, beta-sitosterol, tannins and phenolic compounds (Rajan et al., 2015). Also it consists of betulinic acid, D-mannitol,

stigmasterol, alanine, aspartic acid, glutamic acid, serine and pseudo-jujubogenin glycoside (Devishree et al., 2017).It acts as antimicrobial, antifungal, anti-inflammatory, antiepileptic, anthelmintic, antioxidant, anticancer, cytotoxic, antiallergic, neuro-pharmacological (Roodenreys et al., 2002; Chaudhuri et al., 2004).

Ranunculus sceleratus-Commonly known as Cursed Buttercup, in hindi Jaldhaniya, family Ranunculaceae. Ranunculin compound functions as toxic. This plant is very dangerous. Native to southern temperate, tropic, northern hemisphere areas mainly in higher altitude. An annual, along perennial herb (Aslam et al., 2012). These exists in damp terrain, riversides, water areas. It contains fatty acids, organic acids, phenols, aldehydes, flavonoid, tannins, alkaloids, coumarin, essential oils (Sher, 2009), 5-hydroxy tryptamine, apigenin, apigenin 7-O-β-glucopyranosyl-4′-O-α-rhamnopyranoside,tricin (Bhargava et al.,

1965).It helps in blood circulation, cold, swelling, malaria, scrofula, snake or scorpion venom. Acts as antihemorrhagic, neuralgia pains, anti-spasmodic, diaphoretic, vermifacient, anthelmintic (Wang, 1995; Mantle et al., 2000).

***Acalyphaindica*-**commonly known as Indian IndianCopperleaf, in hindiKuppi, Euphorbiaceae family. Exists in fields, wastes places, hotter areas. Annual-monoecious herb, native to Africa, South Africa, India, Sri Lanka (Masih et al., 2011). Active compounds including Tannins,Flavonoids,carbohydrates,Terpenoids, saponins, phenols, Alkaloids. It acts as diuretic,Anti-helmintic, anti-snake venom (Samya et al., 2008) antioxidant (Marwah et al., 2007), anti-inflammatory effects, diuretic, anti-implantation, anti-estrogenic activity, death through infectious diseases. It treats bronchitis, asthma, pneumonia, scabies (Chitravadivu et al., 2009) pneumonia, skin infection, rheumatoid arthritis, stomach pain,bleeding piles, irritations, stabbing pain, wheezing including Kabha factor (Anbukkarasi, 2012).

Materials And Methods

Plant Materials:-Specimens (roots) of *Withaniasomnifera, Terminalia arjuna, Bacopa monnieri, Ranunculussceleratus and Acalyphaindica*were collected from different locations

of Uttarakhand, near Agrakhal and Uttar Pradesh, near surajpur area.

Plants Authentication:-The botanical authentication was performed by Dr. Shailesh Solanki, Head Of The Department of Agriculture, Faculty Of Sciences, Noida International University, U.P.

Extract Preparation:-Plant roots were washed with tap and distilled water and dried in room temperature for 18-24hrs. Roots were powdered in a mixer grinder and kept in paper bag which was stored in air tight containers for a day. Powered material was extracted with Chloroform,Methanol and Ethanol in 1:10 ratio (Handa et al., 2008). The extracts wereboiled at 40^{O}C-60^{O}C, shaken and filtered. The filtrates were then evaporated in hot air oven at 50^{O}C-60^{O}C for overnight then stored at 4^{O}C.

Phytochemical Qualitative Assay:-Various tests were performed according to the Standard methods (Safowara, 1993; Harborne, 1973) which included Tannin, Saponin, Alkaloid, Flavonoid, Terpenoid, Sterols-Steroid, Phenol, Carbohydrate, Quinone,coumarin, Carboxylic acid, Cardiac Glycoside, Ninhydrin.

Microorganisms Selection:-Two Gram negative bacteria *Acinetobactor baumanni*(MTCC 1425),*Pseudomonas aeruginosa* (MTCC 647) and two gram positive bacteria *Bacillus subtilis*(MTCC 441), *Staphylococcus aureus*(MTCC 96), yeast- *Saccharomyces cerevisiae*(MTCC 250)and fungi-*Aspergillus niger*(MTCC 9652)were used for the experiment. Using Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH),Chandigarh, microbes were collected. Four Standard Antimicrobial agents for bacteria, Ciprofloxacin Tablet (BAYER, 100mg), Streptomycin (X-GEN, 1000mg), Tobramycin Injection I.P. (ICON, 80mg/2ml), Azithromycin Tablet (SANDOZ, 250mg) and two Antifungal agents for fungi and yeast, Itraconazole Capsules (CIPLA, 100mg), Fluconazole Capsules (CIPLA, 150mg) were used.

Inoculum Preparation:-The bacterial strains were grown in Luria Bertani Agar media(Himedia, India)- *S. aureus* and *A.baumanni*, Bacillus Differentiation Agar Media(Himedia, India)- *B.subtillis*, Citrimide Agar Media(Himedia, India)- *P. aeruginosa* at 35°C for 24-48h whereas the yeasts, *S. cerevisiae* and fungi, *A. niger* were grown in Potato Dextrose Agar media(Himedia, India), respectively, at 35^{O}C for 24-96h. The stock cultures were maintained at 4°C.

Antimicrobial Activity:-Agar Disc Diffusion Assay:-To study the antimicrobial activity (Furtado and Medieros,1980) of extracts, different media were used for the proper activity of bacteria and fungi. Luria Bertani Agar media for *A.baumanni* and *S. aureus,*Cetrimide Agar media for *P. aeruginosa,* Bacillus differentiation media for *B. subtilis,* Potato Dextrose Agar media for *S. cerevisiae* and *A. niger* were prepared, autoclaved. The dried plant extract (100 mg) were again dissolved in 5 ml of ethanol, sterilized using Millipore filter (0.22 µm) Whatman filter paper No.1 discs (6 mm diameter). 50ul-100ul of bacterial suspension, having $1.6*10^{8}$CFU.ml^{-1} and $1.2*10^{6}$ CFU.ml^{-1} fungi were spreaded. Discs were loaded with 20ul plant extract of concentration (10 mg/ml) on agar plates. Similarly, Filter paper discs loaded with 2ul of 10mg/ml antibiotic solutionused as positive control and respective solvents as negative control. The bacterial plates were incubated at 35°C for 24-48 h, whereas the fungal plates at 35^{O}C for 24-96h. The diameter of the inhibition zone (mm) was measured manually.

Minimum Inhibitory Concentrations-By Agar Disc Diffusion Assay, the plant extracts exhibiting a strong antibacterial and antifungal activity at 10 mg/ml were used to determine their MIC using disk diffusion method. By twofold dilution, different concentrations of plant extract (0.625.1.25, 2.5, 5.0, 10.0 mg/ml) were prepared separately by dissolving 100 mg in 5 ml of ethanol. Similarly, MIC performed for antibacterial and antifungal agents. Media was poured into sterile Petri dishes and spreaded with 50ul-100ul of bacterial and fungal suspensions of the pathogenic strains. The filter paper discs were loaded with 20ul of different concentrations of the effective plant extracts and placed on agar plates. The plates were incubated at 35°C for 24-96h. The inhibition zones were measured against the concentrations of the effective plant extracts.

Statistical Analysis-In triplicates the works were done. The data were recorded as means ± standard deviations (SD). One Way ANOVA was performed on all the statistical data, $p<0.5$ is taken to be significant.

Results And Discussions

Extraction Process

(a) (b) (c)

Figure 1 Sample Root Extracts, where (a) Ethanolic, (b) Methanolic, (c) Chloroform

Figure 1 showed the root extracts of all the five plantwith each of the three solvents performed by maceration technique.As from picture, E-Ethanol, M-Methanol, C-Chloroform, A-Ashwagandha (*Withaniasomnifera*), Ar-Arjuna (*Terminalia arjuna*), B-Brahmi (*Bacopa monnieri*), J-Jaldhaniya (*Ranunculussceleratus*), K-kuppi (*Acalyphaindica*). Different range of colours had been depicted in the eachof the root extracts of five plants with three different solvents, contributing to the presence of various different compoundsand enhancing various different beneficial activities. These various root extracts colours represent highly potential values.

Qualitative Phytochemical Analysis

Table1 Qualitative Analysis of Ethanol, Methanol, Chloroform Plants Extracts

S.No	Tests	AE	ArE	BE	JE	KE	AM	ArM	BM	JM	KM	AC	ArC	BC	JC	KC
1.	Alkaloid Wagner's test	+ + +	+ + +	+	++	++	+ + +	+ + +	+	+	++	+	+	+	+	+
2.	Tannin	++	+ + +	+	++	++	+ +	+ + +	+	++	++	-	-	-	-	-
3.	Carbohydrate Fehling's Test	++	++	+	++	+	+ + +	+ +	+	+	++	+	+	+	+	+
4.	Flavonoid	+ + +	++	+	++	+++	+ ++	+++	++	++	++	+	-	-	-	-
5.	Saponin	++	++	+	++	++	++	++	+	+	++	-	-	-	-	-
6.	Ninhydrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7.	Phenol	+ + +	+ + +	+ +	++	++	+ + +	+ + +	+ +	+	+++	+	-	-	-	-
8.	Carboxylic Acid	++	+	+	++	++	++	+ +	+	+	++	-	+	+	-	+
9.	Cardiac Glycosides	++	+ +	+	++	++	+ +	+	+ +	++	+	+	+	+	+	+
10.	Coumarins	+ +	+ + +	+ +	++	++	+ + +	+ + +	+	+	++	+	-	+	-	-
11.	Triterpenoids	+	+ + +	+	++	++	+ +	+ + +	+	++	++	-	-	-	-	-
12.	Steroids-Sterols.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Represents Phytochemical analysis of five Plants Extracts, where E-Ethanol, M-Methanol, C-Chloroform, A-Ashwagandha, Ar-Arjuna, B-Brahmi, J-Jaldhaniya, K-kuppi. Indications Shows (-) Absent, (+) Slightly Present, (++) Moderately Present, (+++) Highly Present.

The present study of qualitative analysis of all the five plant's roots extracts in each of the three solvents were shown in Table.1. It revealed the presence of valuable compounds. The indications were based upon the intensity of colour of all the extracts containing respective solvents.Preliminary qualitative phytochemical analysis of roots ethanolic and methanolic extracts of *Withania somnifera*, *Terminalia arjuna*, *Ranunculus sceleratus* and *Acalypha indica* has shown the high presence of flavonoids, phenols, protein triterpenoids,

carbohydrates, alkaloids, tannin, saponin, sterols, carboxylic acids, cardiac glycosides and coumarin. Hence, these have broad-spectrum bioactive in nature. Extracts of *Bacopa monnieri* had the presence of compound in a considerate level. Chloroform extracts showed presence of active compounds at a very low level. Compared to all other solvent extracts, methanolic and ethanolic root extracts had higher number of secondary metabolites with high degree of precipitation (++),(+++).

These secondary metabolites has been reported to have many different biological and therapeutic utilities, expected to have various medicinal uses. Because methanolic and ethanolic solvents has high polarity which is used draw potential variety of plant constituents than other solvents. The phenolic compounds which are found in medicinal plants are mainly highly responsible for a wide range of biological activities, which includes antimicrobial action. Various studies showed the antimicrobial action of phenolic compounds acts by bactericidal effect. Flavonoids is an essential polyphenols, group of heterocyclic organic compounds consisting of important biological properties as antimicrobial potential. Alkaloids on the other hand is another nitrogen based beneficial compound showing antibiotic properties (Bastos et al., 2009).

Antimicrobial Activity- Agar Disc Diffusion Assay

Five plant species were investigated against bacteria including two strains of Gram positive bacteria (*B. subtilis* & *S. aureus*), two strains of Gram negative bacteria (*E. coli, P. aeruginosa*) and *A. niger, S. cerevisiae* by disc diffusion method. The Antibiotic susceptibility screening of pathogens determined by commercial antibiotics by disc diffusion method. It was seen that potentially high antimicrobial activity i.e. high inhibition activity has been shown by ethanolic and methanolic root extracts of*Withaniasomnifera, Terminalia arjuna, Acalyphaindica*against the four bacteria, fungi and a yeast, in Graph.1 at 10mg/ml. It was analyzed that flavonoids, phenols, alkaloids, tannins and some other phenolic compounds are mainly used for their antimicrobial activity (Bastos et al., 2009).Chloroform extracts has shown almost no activity of inhibition against all the microbes. Due to the very low concentration of bioactive compounds present in extracts,it was suggested, a bit more polar solvents are effective to extract organic and inorganic compounds than less polar solvents (Eloff, 1998). Ethanolic and methanolicroot extracts of *Bacopa*

monnieri has shown intermediate activity. There may be lesser active compounds extracted during the extraction method or due to some environmental conditions. The activity differed due to the particular concentration 10mg/ml as, in different concentrations, these extracts had good activity. Ethanol extracts of *Ranunculussceleratus* has shown optimum inhibition activity, as compared with methanol extracts. As compared with Antibacterial and Antifungal agents, ethanol and methanol extracts of five plants has shown better microbe growth inhibition as well as when compared with other root extracts, collected from different places (Saidulu et al., 2014). Hence, Minimum Inhibitory Concentration of these extracts with antibacterial and antifungal agents were conducted.

Many studies highlight the potential of natural compounds against pathogens that cause severe infections from Gram positive bacteria *S. aureus* (MTCC 96), *B.subtilis* (MTCC 441), Gram negative bacteria *A. baumannii*(MTCC 1425), *P. aeruginosa* (MTCC 647), as well as fungi *A. niger* (MTCC 9652) and Yeast *S. cerevisiae* (MTCC 250).The activity is quite different as Gram-positive bacteria consists of rough peptidoglycan cell wall, which is mechanically strong and resistant (Nikolaidis et al., 2014). Whereas in Gram-negative bacteria, consists of second membrane, mainly the outer membrane, acts as an effective barrier. Consequently, these has become resistant to various antibiotics demanding some effective pharmacological alternatives. Antibiotic resistance is one of the global issue in 20th century in developed and developing countries challenging a huge healthcare sectors. The new noble antimicrobial compounds produces a variety of potential bioactive compounds of therapeutic beneficial. There is a high increase in microbial infectious diseases and hence, complications are all over the world mainly due to microbial inhibition resistance.

Minimum Inhibitory Concentrations-By using Disc Diffusion method, from Graph 1it was noted that both ethanol and methanol extracts were comparatively had shown low MIC values against bacteria, fungi and yeast than the Agents.The MIC values were calculated in ug/ml. In *B. subtilis*, the MIC values of methanol extracts of *Bacopa monnieri* (156.5±1.24) and *Acalypha indica* (157.6±1.57) were the low MIC values, showing good inhibition followed by other extracts. In *P. aeruginosa*, ethanol extracts of *Terminalia arjuna* (21.02±1.03) and methanol

extracts of *Withania somnifera* (8.55±0.125) showed lowest MIC values. Ethanol and methanol extracts of *Acalypha indica* (64.10±2.00, 38.69±1.56 respectively) has shown good inhibition effect from other extracts in *A. baumanni*. Ethanol extract of *Terminalia arjuna* (12.32±0.28) has been most effective with very low MIC values in *S. aureus*. In case of *A. niger* and *S. cerevisiae*the MIC values of extracts varied from 475ug/ml-850ug/ml.As compared, MIC values of extracts, ranging from 8.55ug/ml-845.5ug/ml were found to be better, showing good inhibition effects than the antibacterial (588-991ug/ml) and antifungal (850-900ug/ml) agent's MIC values.

In table 2 the concentration effect with Zone of inhibition(mm) of the ethanol and methanol plant extracts were seen. It was noted that most of the ethanol and methanol plant's extracts were started effective inhibition effects from 0.625 mg/ml and it increased and stabilisedat 10mg/ml against bacteria except ethanol and methanol extracts of *Bacopa monnieri* and *Ranunculus sceleratus* showing effects from 1.25mg/ml. In case of fungi and yeast, inhibition startedfrom1.25-2.5 mg/ml. The antimicrobialand antifungal agents showed inhibition effects from 1.25 mg/ml and 2.5mg/ml respectively, increased and stabilised at 10mg/ml.

Table 2 Minimum Inhibitory Concentration Of Ethanol And Methanol Plant's Extracts

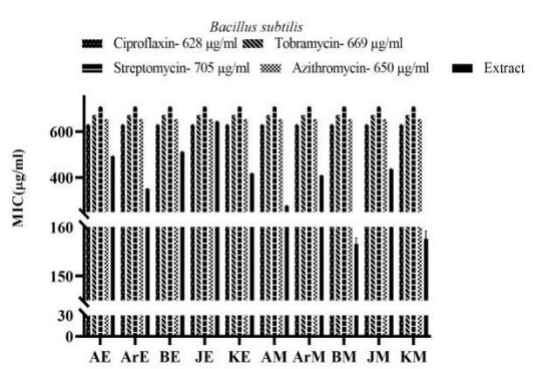
	Bacillus subtilis	***Pseudomonas aeriginosa***	***Acinetobactor baumannii***	***Staphylococcus aureus***	***Saccharomyces cervisiae***	***Asperigillus niger***
E/Ab	**Extract or Antibiotics Concentrations (mg/ml): Zone of Inhibiton±Std.dev(mm)**					
AE	**10**: 24.67±0.57 **5**: 25 **2.5:** 17±1.73 **1.25**: 15 **0.625**: 14.33±0.57	**10:**17.33± 0.57 **5**:15.33 ± 0.57 **2.5:**15 **1.25**:14 **0.625**:13.67 ± 0.57	**10**:26.67 ±0.57 **5**:25 ± 1 **2.5**:20 **1.25**:15.33 ± 0.57 **0.625**:15	**10**:19.33 ± 0.57 **5**:17 ± 1.7 **2.5**:15.67± 0.57 **1.25**:15.33 ±0.57 **0.625**:14	**10**:21.33 ± 0.57 **5**:21.33 ± 0.57 **2.5**:16 ± 1 **1.25**:14.67 ± 1.52 **0.625**:0	**10:**20± 1 **5:**17 ±1.73 **2.5:**16.33 ± 0.57 **1.25:**14.33 ± 0.57 **0.625:**0
ArE	**10:** 23.67±0.57 **5:** 24 **2.5:** 17±1 **1.25:** 15.66±0.57 **0.625:**	**10:**16.33± 0.57 **5:**15.67 ±0.57 **2.5:**15 **1.25:**14.33 ±0.57	**10:**24 **5:**22.67± 1.53 **2.5:**20 **1.25:**15.33 ±1.58 **0.625:**14.33	**10:**17.67 ± 0.57 **5:**16.66 ±0.57 **2.5:**16.33± 0.59 **1.25:**15 **0.625:**14.67	**10:**22.33 ± 0.57 **5:**22.33± 0.57 **2.5:**17.67 ± 0.57 **1.25:**14.67 ± 1.15	**10:**20.67 ± 1.15 **5:**16.67 ± 0.58 **2.5:**17 ± 1 **1.25:**15.33 ± 0.57

	14.66±0.57	**0.625:** 13.67± 0.57	± 0.57		**0.625:**0	**0.625:**0
BE	**10:**13.67±0.57 **5:** 13.33±0.5 **2.5:** 11.33±0.57 **1.25:** 11 **0.625:** 0	**10:**11.33± 0.57 **5:**11 **2.5:**11± 1 **1.25:**10.67 ± 0.57 **0.625:**0	**10:**11.67 ±0.57 **5:**11.67± 0.57 **2.5:**12 **1.25:**11 **0.625:**0	**10:**11.67 ±0.57 **5:**11.33± 0.57 **2.5:**11.33 ± 0.57 **1.25:**11 **0.625:**0	**10:**12 **5:**11.67± 0.57 **2.5:**11.33 ± 0.57 **1.25:**0 **0.625:**0	**10:**12.33 ± 0.57 **5:**11.67 ± 0.57 **2.5:**12.33 ± 0.57 **1.25:**0 **0.625:**0
JE	**10:** 21±1.73 **5:** 21±1.73 **2.5:** 15.33±1.15 **1.25:** 13.66±0.57 **0.625:** 0	**10:**16.67± 0.57 **5:**17± 1 **2.5:**15.67 ± 0.57 **1.25:**13.33± 0.57 **0.625:** 0	**10:**17.67± 0.57 **5:**16.33± 0.57 **2.5:**16 **1.25:**14.67 ± 0.57 **0.625**:0	**10:**18 ± 1 **5:**18 ± 1 **2.5:**15.33 ±0.57 **1.25:**14.33 ± 0.57 **0.625:**0	**10:**19.33 ± 0.57 **5:**17.67± 0.57 **2.5:**15.67 ± 0.57 **1.25:**0 **0.625:**0	**10:**18 **5:**18± 1 **2.5:**14.33± 0.57 **1.25:**0 **0.625:**0
KE	**10:** 22.67±1.15 **5:** 20.6±1.5 **2.5:** 15.66±0.57 **1.25:** 14.33±0.57 **0.625:**14	**10:**16.67 ±1.15 **5:**16 ± 1.73 **2.5:**15± 1 **1.25:**13.67± 0.57 **0.625:** 12.67 ± 0.57	**10:**18.67± 0.57 **5:**17.33 ± 0.57 **2.5:**16.33± 0.57 **1.25:**16.33 ± 0.57 **0.625:**14	**10:**19 ± 1 **5:**19± 1 **2.5:**15 **1.25:**14.33± 0.57 **0.625:** 14	**10:**17.33± 0.57 **5:**17 **2.5:**15 **1.25:**0 **0.625:**0	**10:**18 **5:**15 **2.5:**15.33± 0.57 **1.25:**0 **0.625:**0
AM	**10:** 22.67±2.3 **5:** 21.33±2.3 **2.5:** 18±1 **1.25:** 16 **0.625:**15±1	**10:**16 ± 1 **5:**15.33± 0.57 **2.5:**14.67 ± 0.57 **1.25:**14.33± 0.5 **0.625:** 14	**10:**20.33± 0.57 **5:**19± 1.73 **2.5:**19 **1.25:**14.67± 0.57 **0.625:** 14.33 ± 0.57	**10:**18.67 ± 0.57 **5:**18.67± 0.57 **2.5:**15.67 ± 0.57 **1.25:**14.33± 0.57 **0.625:**13	**10:**19.67± 0.57 **5:**19± 1 **2.5:**16.67 ± 0.57 **1.25:**14.67 ± 0.57 **0.625:**0	**10:**20.33 ± 0.58 **5:**18.67 ± 0.57 **2.5:**18.33± 0.57 **1.25:**14.67 ± 0.57 **0.625:**0
Ar M	**10:** 22.33±1.52 **5:** 22.33±1.5 **2.5:** 16.33±1.15 **1.25:** 14.66±0.57 **0.625:** 14	**10:**15.67 ±0.57 **5:**15.67 ±0.57 **2.5:**15 **1.25:**13 **0.625:**13.33 ± 0.57	**10:**21.67 ± 0.57 **5:**19.33 ± 0.57 **2.5:**17 **1.25:**17 **0.625:**13.33 ± 0.57	**10:**19.67 ± 0.58 **5:**19.33 ± 0.57 **2.5:**15.33± 0.57 **1.25:**14.33± 0.57 **0.625:**14	**10:**21.67± 1.15 **5:**21.33± 1.52 **2.5:**17.67± 0.57 **1.25:**16 **0.625:**0	**10:**18.67± 0.57 **5:**18.33± 0.58 **2.5:**18 ± 1 **1.25:**15 **0.625:**0
BM	**10:** 12 **5:** 11.66±0.57 **2.5:** 12 **1.25:** 0 **0.625:** 0	**10:**11.33± 0.57 **5:**10.67± 0.57 **2.5:**11.6 **1.25:**11.33±	**10:**11 **5:**11.67± 0.57 **2.5:**11.67 ±0.57 **1.25:**11±	**10:**11.67 ± 0.57 **5:**11.33 ± 0.57 **2.5:**11 **1.25:**11 **0.625:**0	**10:**12.33± 0.57 **5:**11 **2.5:**12.33± 0.57 **1.25:**0	**10:**12 **5:**11.67± 0.57 **2.5:**11.67 ± 0.57 **1.25:**0

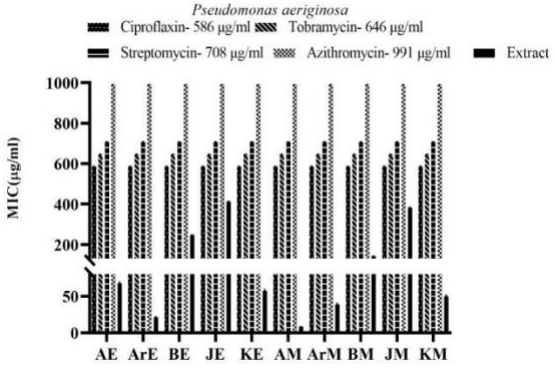
		0.57 **0.625:** 0	0.57 **0.625:**0		**0.625:**0	**0.625:**0
JM	**10:** 13.67±0.57 **5:** 14±1 **2.5:** 13±1 **1.25:** 11 **0.625:** 0	**10:**13.67± 0.57 **5:** 13.67 ±0.57 **2.5:**12 **1.25:**12 **0.625:**0	**10:**14 **5:**14 ± 1 **2.5:**13.67± 0.57 **1.25:**13.33± 0.57 **0.625:**0	**10:**13.67± 0.58 **5:**13.33 ± 0.58 **2.5:**13 **1.25:**13 **0.625:**0	**10:**14 **5:**13.67± 0.57 **2.5:**13.33± 0.57 **1.25:**0 **0.625:**0	**10:**14 **5:**13.67 ± 0.57 **2.5:**14 ± 1 **1.25:**0 **0.625:**0
KM	**10:** 18.67±1.15 **5:** 18 **2.5:** 16.66±0.57 **1.25:** 14.33±0.5 **0.625:** 14	**10:**16.67± 0.57 **5:**15.67 ± 1.15 **2.5:**14.67 ± 0.57 **1.25:**14.33± 0.57 **0.625:**13.33 ± 0.57	**10:**17.33± 0.57 **5:**16.67 ± 0.57 **2.5:**16.33± 0.58 **1.25:**15.33± 0.57 **0.625:**13.67 ± 0.57	**10:**17.67 ± 0.57 **5:**16.67 ± 0.67 **2.5:**16± 1 **1.25:**14 **0.625:** 13.67 ± 0.57	**10:**20 ± 1 **5:**18± 1 **2.5:**15± 1 **1.25:**0 **0.625:**0	**10:**15.67 ± 0.57 **5:**15.33 ±0.58 **2.5:**14.33± 0 .51 **1.25:**0 **0.625:**0
Ci	**10:** 39.67±0.57 **5:** 37.33±0.1 **2.5:** 37.2±1.15 **1.25:** 11.66±0.52 **0.625:** 11.33±0.57	**10:**44.67± 0.24 **5**:44.67 ± 0.13 **2.5:**44.33± 0.34 **1.25:**11.33 ± 0.12 **0.625:** 11	**10:**39.73 ± 0.21 **5:**38.73 ± 0.21 **2.5:**39± 1 **1.25**:11.67± 0.21 **0.625:** 11.33 ± 0.21	**10:**39.73 ± 0.23 **5:**39.33± 1.15 **2.5:**38.67 ± 1.13 **1.25:**11.67± 1 **0.625:**11.33± 1	-	-
T	**10:** 14.67±1.15 **5:** 14.3±1.15 **2.5:** 13±1 **1.25:** 8.33±0.57 **0.625:** 0	**10:**14.33 ±0.56 **5:**13.67± 0.45 **2.5:**13.33± 1.15 **1.25:**8.33 ± 0.57 **0.625:**0	**10**:14.33± 0.12 **5:**14± 1 **2.5:**13.33 ± 1.25 **1.25:**8.67 ±1.15 **0.625:**0	**10:**14.33 ± 0.57 **5:**14.33± 0.13 **2.5:**12.67 ± 0.27 **1.25:**9.33 ±0.14 **0.625:**0	-	-
S	**10:** 13 **5:** 13 **2.5:** 12 **1.25:** 0 **0.625:** 0	**10:**13 ± 1.15 **5:**13 **2.5:**12 **1.25:**0 **0.625:**0	**10:**13± 1 **5:**13 ± 1 **2.5:**11 **1.25:**0 **0.625:**0	**10:**13 **5:**13 **2.5:**11.67 ± 1.15 **1.25:**0 **0.625:**0	-	-
Az	**10:** 11.67±1.23 **5:** 11.67±0.57 **2.5:** 11 **1.25:** 0 **0.625:** 0	**10:**11.33± 0.2 **5:**11.33± 0.23 **2.5:**0 **1.25:**0 **0.625:**0	**10:**11.67 ± 0.57 **5:**11.67 ± 0.13 **2.5:**11.33 ± 0.12 **1.25:**0	**10:**11.33± 0.31 **5:**11.67 ± 0.57 **2.5**:11.67± 1.15 **1.25:**0 **0.625:**0	-	-

			0.625:0			
F	-	-	-	-	**10:**14.33 ± 0.57 **5:**14 ± 1 **2.5:**10.67± 0.57 **1.25**:0 **0.625:**0	**10:**14.33 ±0.57 **5:**14.33± 0.58 **2.5:**10 ±1 **1.25:**0 **0.625:**0
I	-	-	-	-	**10:**14 ±1 **5:**14 ±1 **2.5:**9.67± 0.57 **1.25:**0 **0.625:**0	**10:**13 **5:**13 **2.5:**10± 1 **1.25:**0 **0.625:**0

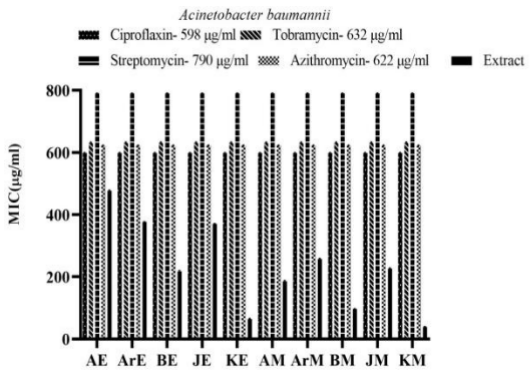
It depicts inhibition distance (mm) of five plants ethanol, methanol extracts and standard antibacterial and antifungal agents against bacteria, fungi and yeast. E- Extracts, Ab- Antibiotics, Ci- Ciprofloxacin, T- Tobramycin, S- Streptomycin, Az- Azithromycin, F- Fluconazole, I- Itraconazole.

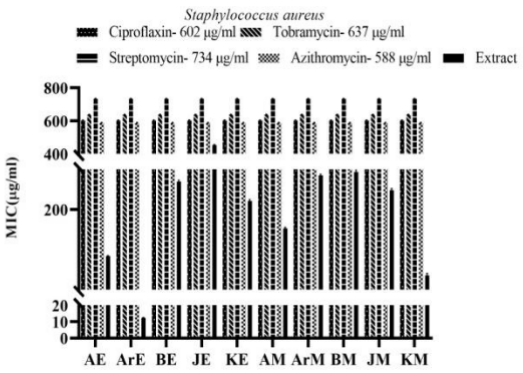


(a)

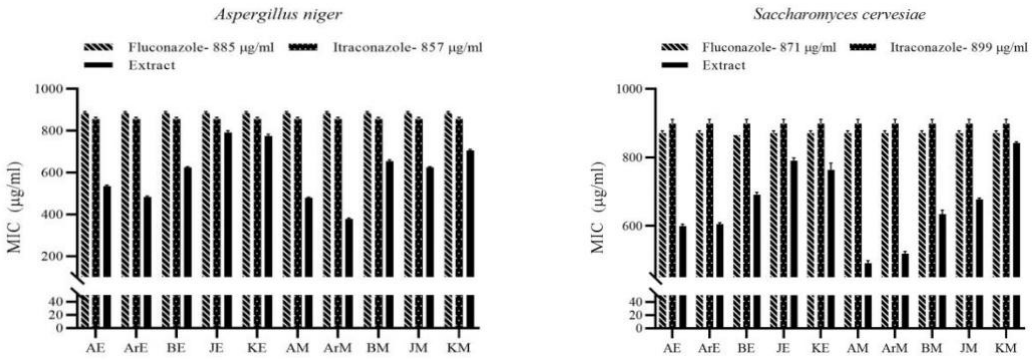


(b) **(c)**





(d)



(e) **(f)**

Graph.1 Comparative graph of the Minimum Inhibitory Concentration (MIC) of ethanolic, methanolic plant's extracts and antibiotics against bacteria and fungi and yeast. Significance with P<0.0001, One Way Anova. Indications shows (a)*Bacillus subtilis*, (b)*Pseudomonas aeruginosa*, (c) *Acinetobacter baumannii*, (d)*Staphylococcus aureus*, (e)*Aspergillus niger*, (f) *Saccharomyces cerevisiae*.

Minimum Inhibitory Concentration, it was reported that these root ethanol and methanol extracts were enhancing better microbial inhibition functions at low MIC values than that of the Standard Antimicrobial agents. As compared with other root extracts (Sundaram et al., 2011; Aneja et al., 2012)collected from different places. Whereas Ciprofloxacin showed appropriate performance at 0.625mg/ml. Ciprofloxacin, a fluro-quinolones was seen to have huge antimicrobial action as the structure compounds shows different spectral activity and pharmacokinetic profiles. Two essential bacterial enzymes are targeted, DNA gyrase (topoisomerase II) and DNA topoisomerase IV. During antimicrobial process, there is a reaction intermediate containing quinolone enzyme and DNA, leading to the DNA replication blockage (Aldred et al., 2014). In some, within hours bacterial death occurs. Hence, Gram positive bacteria *B. subtilis*, *S, aureus* and Gram negative bacteria *A. baumannii*, *P. aeruginosa*, yeast- *S. cerevisiae*, fungi-*A. niger* were highly resistant to these extracts.

Conclusion

The study reports a rich knowledge of affluent medicinal plants diversity in the areas of Agrakhal and Surajpur enhancing various potential activity. This work revealed high presence of phenolic and flavanoid compounds in these medicinal plants as the ethanolic and methanolic root extracts of these medicinal plants tend to possess potential antimicrobial properties at a huge level against the microorganisms.Phytochemical, Qualitative method revealed the presence of important bioactive compounds in a high concentration in ethanol and methanol

root extracts. Ethanolic and methanolic root extracts of *Withania somnifera, Terminalia arjuna* and *Acalypha indica* showed high antimicrobial activity than *Bacopa monnieri* and *Ranunculus sceleratus*. They showedpotentially better microbial inhibition propertyat low MIC values against bacteria, fungi and yeast as compared to other Standard Antimicrobial and Antifungal agents except Ciprofloxacin. Therefore, the utilisation of natural bioactive constituents is necessary for its beneficial and potential antimicrobial functions against various micro-organisms. These are more effective than the commercial antimicrobial agents.These root extracts, at low MIC contained potent antimicrobial and antifungal activity, which in future can lead to formulation of broad novelantimicrobial spectrum.

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