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PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF *ALLIUM TUBEROSUM*

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ABSTRACT

Medicinal plants are excellent sources of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compound which could be serves as a newer lead and clues for modern drug design. Allium tuberosum has a wide variety of antimicrobial properties which is a fine source in selecting the plant and describing its activity. The method of extraction and methods opted for describing its antimicrobial activity has been done by the use of ethanolic extractions for antimicrobial activity, NCCS method for antibacterial activity and antifungal activity by PDA media. The results obtained shows that B.subtilis shows maximum inhibition when compared with other microorganisms. Antifungal activity by PDA media proves that the *Sclerotium rolfsii* has shown maximum inhibition of 5mm with Nystatin as control. Zone of inhibition of leaves of *Allium tuberosum* has minimum formation of zone at various concentrations such as 10µl, 20µl, 30µl, 40µl using organism namely *Aspergillus flavus, Fusarium verticillodies, Sclerotium rolfsii.* It has been shown that *Fusarium verticillodies* shows maximum inhibition of 14mm with 40 µl of A.tuberosum concentration. The ethanolic extract of leaves of *Allium tuberosum* was inoculated against *K.pneumoniae, P.aeruginosa, S.aureus and B.subtilis* in the concentration of 8-0.25 mg/100µl. The growth of *S.aureus, P.aeruginosa, K.pneumoniae* and *B.subtilis* were inhibited by ethanolic extract of Allium *tuberosum* of 8-1.0 mg/100µl concentration. *S.aureus* and *P.aeruginosa* was found to have significant killing effect at 8-1.0 mg/100µl whereas *K.pneumoniae* has the killing effect at 8-2 mg/100µl concentration. *B.subtilis* found to have killing effect at 8-0.5 mg/100µl.

KEYWORDS: Agar well diffusion method Nystatin Potato dextrose agar media. Reactive oxygen species.

INTRODUCTION

Medicinal plant besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drug with precise selectively. These are the reservoirs of potentially useful chemical compound which could serve as newer leads and clues for modern drug designing **(Vijayalakshmi and Ravindra, 2012)**. The medicinal plants are considered as a rich resource of ingredient which can be used in drug development and synthesis **(Hassan, 2011)**.

Phytochemical and phytopharmacological sciences have already established the composition and biological activities of several medicinal plant products. Most of the biologically active constituents of extracts such as flavonoids, tannins and terpenoids are highly water soluble but demonstrate a low absorption, because they are unable to cross lipid membrane, because of high molecular size, resulting in loss of bioavailability and efficacy **(Bonifacio et al. 2014**). The plant consist of primary and secondary metabolites in which primary metabolites are lead for growth and development of plants secondary metabolites are playing role in defense mechanism against harmful organisms and infectious agents for the plant **(Amabye et. al, 2015).**

Infectious diseases are spread worldwide and in developing countries due cause of morbidity and mortality rate. Antimicrobial drugs are developed by the role of microorganism which is against antimicrobials. Antimicrobial potential of natural products has more important to increase the resistance of certain antibiotics. **(sabbobeh et al, 2016).**

The four major aspect of A.tuberosum is considered to be the inhibition of reactive oxygen species (ROS). The scavenging of free radicals is the prince factors for controlling degenerative or pathological process of various serious ailments in human body such as aging, cancer, Alzheimer's disease and heart diseases. Plant derived antioxidants, such as ascorbic acid, α - tocopherol, polyphenol and flavonoids are becoming increasingly popular as important dietary factors, due to the low risk associated with them. A. tuberosum, as high medicinal value herb, is very potent source of several antioxidants **(Sultana et al, 2015).**

METHODOLOGY

The collected samples were authenticated by botanical survey of India, Coimbatore. The authentication number is BSI/SRC/5123/2014-2015/Tech 510.

Preparation of the extract: The fresh leaves of the Allium tuberosum were taken and cut into small pieces and the 10g of leaves were weighed and ground with the solvents (methanol, aqueous, chloroform, petroleum ether). Then filtered.

PHYTOCHEMICAL ANALYSIS

The leaves of Allium tuberosum were indicated by using phytochemical screening of various tests such as sugar, proteins, flavonoids, phenols, terpenoids, were carried out using petroleum ether, chloroform, methanol, and aqueous extracts.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of ethanolic extracts of Allium tuberosum was determined using bacterial culture namely Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis. The fungi are Aspergillus flavus fusarium verticillodies and Sclerotium rolfsii. The strains of the bacterial culture were bought from microlab, Coimbatore. This was stored at 4^0C and use for further experiment.

ANTIBACTERIAL ACTIVITY

Agarwell diffusion method According to NCCLS method Muller Hinton agar was used as a nutrient source of bacteria. The leaves of Allium tuberosum was dissolved in known amount of dimethyl sulphoxide. 20ml of Muller Hinton agar was poured into the petriplates which was allowed to solidify and the well was cut by gel puncture in the size of 6mm diameter. Then 20µl of sample was loaded and incubated at 37˚C for 24 hrs. The obtained zone of inhibition around the well was measured in millimeters (NCCLS, 2000).

ANTIFUNGAL ACTIVITY

Potato dextrose agar medium was prepared and poured on the petriplates. An extracts were also placed in the plates. Nystatin was used as antifungal control. The antifungal effects was seen as crescent shaped zones of inhibition **(Schlumbaum et.al, 1986)**.

MINIMUM INHIBITORY CONCENTRATION (MIC)

Dilution method used to investigate minimum concentration of antimicrobials to kill or inhibit the microbial growth. Micro titre plates were added with 100µl of nutrient broth and

diluents which makes the well to two folds dilution. From first well 100µl of the dilution was taken and transferred in to the second row well to make a 4:1 dilution. This proceeds sequentially to make each well to 2 fold dilution. After the dilution of the sample, an aliquot of test organism was to all well and the plate was kept for incubation for overnight. Followed by incubation MIC was calculated as the lowest concentration of sample extracts which inhibits the growth of bacteria. If the sample concentration is insufficient the bacterial growth will be seen in the well **(Eloff, 1998)**.

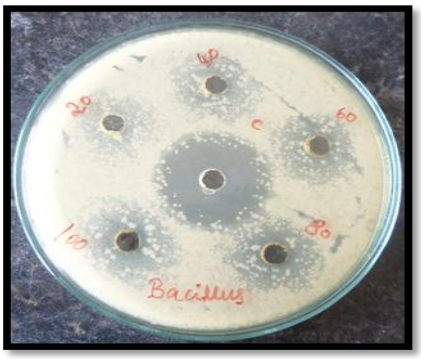
RESULT AND DISCUSSION

Higher plants as a natural source will give rise to a source of antimicrobial agents. The drug is resistance to human pathogen is against particular type of antibiotics. New antimicrobial agents are derived from other sources. Medicinal plants are derived screened for antimicrobial activity to find a new substances for therapeutic purposes **(Kiruthiga et al., 2014).**

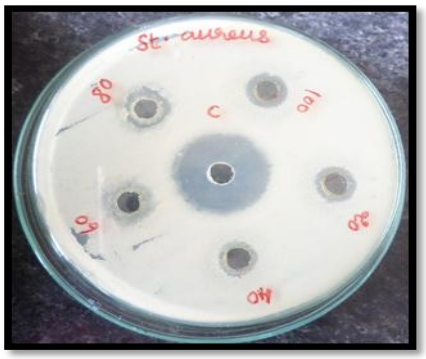
Leaves of Allium tuberosum are dissolved using DMSO. The organism namely Bacillus, Klebsiella, Pseudomonas and Staphylococcus. Antibacterial activity of leaves of Allium tuberosum is treated with gram positive and gram negative bacteria using control as gentamycin. The zone of inhibition is measured by mm.

Table: 1. Zone of inhibition (mm) of ethanolic extract of leaves of allium tuberosum against microorganism.

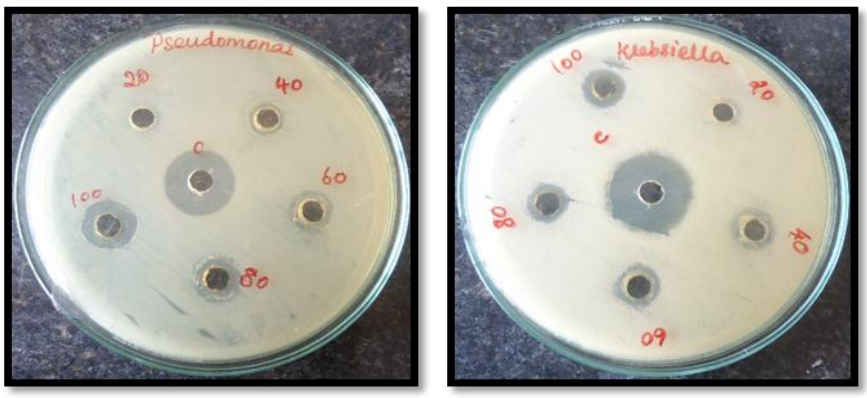
Microorganism	Zone of inhibition (mm)					
	20µl	40µl	60µl	80µl	100µl	Control Gentamycine
Bacillus subtilis	15	20	20	22	23	25
Staphylococcus aureus	9	11	12	12	14	22
Pseudomonas aeruginosa	7	9	10	13	15	17
Klebsiella pneumonia	8	11	12	13	13	20



Bacillus subtilis



Staphylococcus aureus



Pseudomonas aeruginosa **Klebsiella pneumonia**

Plate-1.

Plate 1 confirms that the various concentrations of *Allium tuberosum* leaves extract have effective antibacterial activity against gram positive and gram negative bacteria. From the results, it can be concluded that the zone of inhibition was increased when the concentration of ethanolic extract of leaves of *Allium tuberosum* increased. However the highest zone of inhibition was found against *B.subtilis* (23mm) while compared with other bacterial strains at 100µg/ml concentration followed by *P.aeruginosa* (15 mm), *S.aureus* (14 mm) and *K.pneumoniae* (13mm). The leaves of *Allium tuberosum* exhibited highest zone of inhibition with minimum concentration, which can potentially eliminate the problem of clinical pathogens which may have the adverse effects and its application thus making them more biocompatible.

ANTIFUNGAL ACTIVITY

Antifungal activities of *Melastoma malabathricum* of zone of inhibition showed in mm are maximum for *Aspergillum flavus, Fusarium verticillodies, Sclerotium rolfsii* (Mirshra *et al.*, 2015).

Zone of inhibition for the leaves of *Allium tuberosum* was observed using nystatin as control showed minimum zone of inhibition in organism namely *Aspergillus flavus*- 3mm, *Fusarium verticillodies*-2mm *and Sclerotium rolfsii*-5mm.

Zone of inhibition of leaves of *Allium tuberosum* has minimum formation of zone at various concentrations such as 10µl, 20µl, 30µl, 40µl using organism namely *Aspergillus flavus, Fusarium verticillodies, Sclerotium rolfsii* which are shown below.

Table: 2.Antifungal activity of *Allium tuberosum* using control zone of inhibition in mm.

Fungi organism	Nystatin (5µl)
Aspergillus flavus	3mm
Fusarium verticillodies	2mm
Sclerotium rolfsii	5mm

Antifungal activity of *Allium tuberosum* was found to be more which has minimum zone of inhibition whereas other leaves like *Achillea biebersteinii* are less due to lack of zone in the organisms.

Zone of inhibition are maximum in 40µl of concentration in *Fusarium verticillodies*, other organism produced zone of inhibition at minimum level. So, *Fusarium verticillodies*, is used to indicate the presence of broad spectrum antibiotic compounds and therefore the leaves of *Allium tuberosum* could be used to identify the new drugs to control the growth of the fungi.

Table: 3. Antifungal activity of *Allium tuberosum* using different concentration.

Fungi organism	10µl	20µl	30µl	40µl
Aspergillus flavus	5mm	6mm	7mm	9mm
Fusarium verticillodies	6mm	9mm	10mm	14mm
Sclerotium rolfsii	8mm	9mm	11mm	13mm

Aspergillus flavus

Fusarium verticillodies

Sclerotium rolfsii

Table: 4. ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *Allium tuberosum* BY MINIMUM INHIBITORY CONCENTRATION (MIC) METHOD.

Micro organisms	Concentration of organisms (µg/ml)						
	0	8	4	2	1	0.5	0.25
Bacillus subtilis	-	+	+	+	+	+	-
Staphylococcus aureus	-	+	+	+	+	-	-
Pseudomonas aeruginosa	-	+	+	+	+	-	-
Klebsiella pneumoniae	-	+	+	+	-	-	-

(+) - Inhibition of microorganism growth (-) - Growth of microorganism

The ethanolic extract of leaves of *Allium tuberosum* was inoculated against *K.pneumoniae*, *P.aeruginosa*, *S.aureus and B.subtilis* in the concentration of 8-0.25 mg/100µl. The growth of *S.aureus, P.aeruginosa, K.pneumoniae* and *B.subtilis* were inhibited by ethanolic extract of Allium *tuberosum* of 8-1.0 mg/100µl concentration. *S.aureus* and *P.aeruginosa* was found to have significant killing effect at 8-1.0 mg/100µl whereas *K.pneumoniae* has the killing effect at 8-2 mg/100µl concentration. *B.subtilis* found to have killing effect at 8-0.5 mg/100µl.

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