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Chemical Diversity Meets Microbial Defense: Antibacterial and Antifungal Efficacy of *Tagetes Minuta* Plant Extracts

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Abstract

Medicinal Plants have been utilized for thousands of years to treat health complications and to inhibit disease triggering agents. The awareness of the medicinal plants curative potential has been transferred over the centuries within and among the human societies. As a consequence of drug resistance and the side effects of medications, the research approach is progressively concentrated on the usage of natural resources, so this manuscript explore novel findings associated to the photochemistry and antimicrobial activities of bioactive moieties from the *Tagetes minuta* species. It frameworks the antimicrobial activities of *Tagetes minuta* species in relative to drug finding. *Tagetes minuta* species are effective medicinal plants utilized in traditional medicine. They have conventionally been employed to treat different complications such as diaphoretic, purgative, menstrual stimulant. In the current study *Tagetes minuta*, a prominent member of the *Asteraceae family* was investigated for in-vitro antimicrobial activity against different strains of bacteria by applying disc diffusion scheme. *Tagetes minuta* extracts was found to have inhibitory effects against the bacterial strains under observations. It was established that *Tagetes minuta* showed an inhibitory result on the bacteria under study. The present research suggests a precise contextual for the substantial application of plant under study for the discourse of numerous pathological infections. *Tagetes minuta* extracts contain a substantial amount of antibacterial and antifungal components, most of which are potent and have varied degrees of inhibitory activity. Ethanoic extract was more efficacious in range of plant extracts, followed by methanol, ethyl acetate, hexane and aqueous extracts.

KEYWORDS

Antimicrobial activity, Medicinal plants, Photochemistry, Plant extracts, *Tagetes minuta*

1. Introduction

Pharmacological and scientific organizations have recently recognized the importance of therapeutic herbs, as well as several publications have acknowledged the positive potential of natural molecules to authenticate the benefits of their biologically active compounds [1].

Phytochemicals are biologically effective, naturally existing chemical compounds found in plants, which contribute health assist for humans [2]. Plants play a noteworthy part in preventing and treating human diseases [3]. People have been using plants as traditional medicine for centuries [4]. Plants are recognized as rich sources of phytochemical components which assist to have medicinal importance [5]. The increasing pervasiveness of antibiotic-resistant pathogens, has made it essential to substitute alternate sources of antimicrobial products [6]. There is an excessive attention on reviving herbal medicine [7] and assimilating their usage into contemporary medicine because of their low cost, drug resistance ability, medicinal value and cultural exchange [8]. Although various assistances can be gained from the usage of herbal plants, probable areas of the interest comprise: probable potential toxicity and unknown drug interactions [9]. The mechanisms behind herb-drug interactions are not completely known, however both pharmacokinetic and pharmacodynamics processes may be important. [10]. Plant extracts are usually efficient organic molecules that work with enhanced types of nutrients and fibers to produce an integrated constancy of the defense system in conflict with different stress situations and disorders [11]. Exploring the therapeutic properties of plants is a time of life practice. Plant-derived compounds have lately gained popularity due to their numerous uses [12]. The use of bioactive molecules from medicinal plants as medicinal beneficial agents has been a significant area in natural product exploration [13]. In Pakistan, the medicinal plants are commonly used in rural areas as compare to urban. The usage of such substitute medicinal plants befalls in numerous procedures for the cure of diverse forms of diseases [14]. *Tagetes minuta* (Fig 1) is an established *Asteraceae* family member, Tagetes glandulifera is a synonym. It has small involucres with a distinct smell and poisonous blooms. It is a weed that can grow in nearly every

temperate zone on the biosphere [15]. This plant is more common inhabitants of South America's mountainous areas, temperate grasslands and Asia. *Tagetes minuta* is also distributed in Kenya, Ethiopia, India, Spanish, and East Africa [16]. This plant may survive in a variety of climatic conditions in Pakistan, including elevations ranging from 3000 to 11000 feet above the sea level in territories of the world. Tagetes minuta favors fresher environments and is prevalent in Pakistan's especially in Hazara and Swat areas. A phytochemical analysis of the plant reveals saponins, tannins, terpenoids, flavonoids, and alkaloids [17]. This plant is a diuretic, stomach tonic, diaphoretic, purgative, menstrual stimulant, and hysteria curative [18]. Plants have the inestimable capability to manufacture aromatic compounds, phenols and their oxygen synthetic analogues are among the most abundant. These compounds have antibacterial properties and are used by plants to defend themselves against harmful microbes [19].

Fig 1. Image of *Tagetes minuta*

2. Material and methods

The antibacterial activity of *Tagetes minuta* Phytoconstituents in ethanol, methanol, ethyl acetate, aqueous, and hexane was investigated. In dimethyl sulfoxide solvent, extracts of the whole plant were calibrated to 1 milligram/6 microliter. Stock solution was made; each 6 microliter of solution contains one milligramme of extract.

2.1. Methods of plant extract preparation

The extraction approach includes the separation of pharmacologically active plant tissue from passive moieties utilizing specific solvents and extraction processes. Solvents infiltrate the earth and plant tissues, dissolving substances of equal polarity. The nature of bioactive compound is determined by various variables such as solvent selection, plant material, and extraction process [20].

2.2. The extraction methods

The timeframe of the extract concentration, solvent usage, solvent pH, temperature, particle density of the plant tissues, and the sample-to-solvent ratio are all factors that influence extraction process. The essential premise is to finely grind the whole plant matter (dry), thereby expanding the area of the extraction and so speeding up the extraction process [21]. The dehydrated entire plant was ground to fine particulates in a grinder, then putting in a precise quantity of solvent and strenuously shaken for 5-10 minutes before being left for 24 hours. The extract was carefully filtered, then the filtrate was dried under reduced pressure and based on the view in the solvent to determine the concentration.

2.3. Choice of solvents

The sort of solvent used during the separation process has a substantial influence on the successful identification of biologically active moieties from bioactive compound. Low toxicity, capacity to evaporate at low temperatures, preservation effect, and inability to enable the extract to combine or dissociate are all characteristics of a suitable solvent in plant extraction methods. Because the final outcome of extraction will contain leftover solvent components. The solvent employed is also controlled by the chemicals to be extracted. The bulk is generally used to assess plants for potential medicinal qualities, accompanied by different organic solvents such as ethanol, methanol, ethyl acetate, hexane, and aqueous.

2.4. Disc Diffusion Method

In this research, the disc diffusion technique has been employed for antimicrobial exploration. To search the existence of

antibacterial fractions in the plant extracts under investigation, a bacterial culture was altered to 0.5 turbidity McFarland principles. Petri dishes were withered for 0.25hr before being employed for antimicrobial analysis. The disc that was plunged with extracts from *Tagetes minuta* were used on the agar Mueller-Hinton. Each test plate comprises of four discs. One for standard antibiotic as a positive control disk (commercial antibiotic), one for negative control and two treated disks, samples. The positive control discs were Ciprofloxacin and Azithromycin. The plates were then kept at 37°C for 18 to 24 hours. After incubation, the plates were inspected for the zone of inhibition.

3. Result and Discussion

3.1. Antimicrobial activity of *Tagetes minuta*

Tagetes minuta plant extracts in five distinct solvents, namely ethanol, hexane, ethyl acetate, methanol, and water have been tested against diverse kinds of bacteria and fungus in the present research.

3.2. Use of standard drug against different bacteria and fungi

Ciprofloxacin, a common antibiotic, was used to treat three Gram-negative bacteria, *Escherichia coli* (Fig 3), *Salmonella typhi*, and *Klebsella pneumonia*, and *Three Gram-positive bacterial strains*, *Staphylococcus aureus, Bacillus subtilis, and Bacillus atropoeus*, *were cured with azithromycin* whereas Coltrimazole was administered to treat Candida albicans, Aspergillus fumigatus. Azithromycin inhibited Bacillus subtilis, Staphylococcus aureus, and Bacillus atropheous at 50mm, 48mm, and 65mm. Ciprofloxacin evidenced antimicrobial properties against Salmonella typhi, Escherichia coli, and Klebsella pneumonia of 40mm, 35mm, and 45mm, respectively, because although Coltrimazole proven antibacterial property against Candida albicans, Aspergillus, and Rhizopus of 25mm, 65mm, and 60mm, respectively.

3.3. Different solvents extract of *Tagetes minuta* plant against different microbes

Ethanolic extract of *Tagetes minuta* exhibited improved inhibitory potential against with the Gram positive bacterium, B. subtilis (20 ± 0.2 mm zone of inhibition), the Gram negative bacterium, E. coli (18 ± 0.0 mm zone of inhibition, Fig 2), the fungus, C. albicans (22 ± 0.2 mm zone of inhibition), and the controls (standard) outcomes were 50 ± 0.0 mm, 35 ± 0.0 mm,

and 25 ± 0.0 mm, respectively (Table.1 and Graph.1). Methanolic extract showed established inhibition consequence toward Gram positive bacteria S. aureus (14 ± 0.1 mm zone of inhibition), Gram negative bacterium E. coli (15 ± 0.0 mm zone of inhibition), fungus C. albicans (14.5 ± 0.5 mm zone of inhibition), and standards were 48 ± 0.0 mm, 35 ± 0.3 mm, and 25 ± 0.2 mm (Table.1 and Graph.2). Extracts of ethyl acetate of *Tagetes minuta* demonstrated good inhibitory impact against Gram positive bacterium, S. aureus (14 ± 0.1 mm zone of inhibition), Gram negative bacterium, E. coli (15 ± 0.4 mm zone of inhibition), fungus, C. albicans (14.5 ± 0.5 mm zone of inhibition), and controls, with results of 48 ± 0.0 mm, 35 ± 0.4 mm, and 25 ± 0.0 mm, respectively (Table.1 and Graph.3).

Tagetes minuta hexane extract had the strongest inhibitory efficiency against the Gram positive bacteria, S. aureus (16 ± 0.03 mm zone of inhibition), Gram negative bacterium, E. coli (14 ± 0.04 mm zone of inhibition), fungus, C. albicans (19 ± 0.04 mm zone of inhibition), and the controls were 48 ± 0.04 mm, 35 ± 0.06 mm, and 25 ± 0.05 mm, correspondingly (Table.1 and Graph.4). The aqueous extract of Tagetes minuta had the greatest inhibitory activity against the Gram positive bacteria B. subtilis (19 ± 0.04 mm zone of inhibition), Gram negative bacterium E.coli (14 ± 0.07 mm zone of inhibition), and fungus C. albicans (12 ± 0.10 mm zone of inhibition). The results for the controls were 50 ± 0.08 mm, 35 ± 0.06 mm, and 25 ± 0.03 mm (Table.1 and Graph.5).

4. Discussion

Resistance in pathogens is one of the spontaneous physiological and biochemical processes in response to the existence of a disinfectant that suppresses susceptibility organisms while preferring resistant ones. Plants, as a source for therapeutic phytochemicals, have traditionally played a crucial role in contemporary health maintenance. Medicinal plants have the capability to treat bacterial confrontation to numerous kinds of antimicrobial drugs. The searching for these kind of plants' foundational bioactive constituents that can

be employed in the treatments of drug-resistant disorders with antibacterial may be a realistic approach to combating the problem of resistant microorganisms. In order to stop the development of increased emergent and resilient contagious disease, it will need a multi-pronged methodology that contains the advancement of novel medications.

5. Conclusion

Plants contain various bioactive substances and are major sources of novel and physiologically active antibacterial chemicals. The exploration of medicinal plant is important for discovery of novel medicines, but its effectiveness cannot be overstated if the technique is not standardized to get equivalent and reproducible outcome. The extracts of *Tagetes minuta* plant have demonstrated significant antibacterial potential against the tested pathogenic bacteria. At current era, scientists are exploring plant parts in different form for antimicrobial activities. Standardizing plant extraction procedures and in-vitro antimicrobial anticipatory assessment would indeed be useful so that the search for novel biologically active relevant plant moieties should be more systematic and the analysis of the data could be aided. Based on the achieved result it is concluded that *Tagetes minuta* extract showed excellent antimicrobial potency, efficiency order for plant extracts were in order of: ethanol > methanol > ethyl acetate > hexane > aqueous extract and inhibitory effect was in order of: fungi > Gram-negative bacteria > Gram-positive bacteria.

Microbes	Extract Concentration (mg/microliter)	Inhibition zone diameter (mm) + (mean±standard deviation)				
		Ethanolic extract	**Methanolic extract**	**Ethyl acetate extract**	**Hexane extract**	**Aqueous extract**
S. aureus	6 mg/microliter	12.5±0.5	11.5±0.5	12±0.0	12±0.1	5.5±0.0
	12 mg/microliter	14.5±0.5	13±0.1	13±0.0	14±0.1	9.5±0.5
	18 mg/microliter	16±0.0	14±0.1	14±0.1	16±0.0	12±0.1
B. subtilis	6 mg/microliter	13.5±0.0	9±0.0	11.5±0.5	10.5±0.5	10±0.1
	12 mg/microliter	16±0.1	13.5±0.5	15.5±0.5	14.5±0.5	14.5±0.5
	18 mg/microliter	18±0.1	15±0.1	15±0.0	16±0.0	19±0.0
	6 mg/microliter	12.5±0.5	12±0.1	8.5±0.5	9.5±0.5	10±0.0

B. atropoeus	12 mg/microliter	14.5±0.5	14±0.0	13±0.1	13.5±0.5	13±0.1
	18 mg/microliter	20±0.2	16±0.0	16±0.1	15±0.1	15±0.1
E.coli	6 mg/microliter	12±0.2	9±0.1	10±0.0	8.5±0.5	11.5±0.5
	12 mg/microliter	15±0.0	12±0.2	13±0.1	11±0.1	13.5±0.5
	18 mg/microliter	18±0.0	15±0.0	15±0.0	14±0.0	14±0.0
S.typhi	6 mg/microliter	12.5±0.5	11.5±0.5	9±0.0	9±0.0	10.5±0.0
	12 mg/microliter	17.5±0.0	13±0.1	11.5±0.0	10.5±0.0	13.5±0.1
	18 mg/microliter	19.5±0.1	16±0.2	16±0.1	11±0.1	15±0.0
K.Pneumoniae	6 mg/microliter	12±0.2	12±1.5	7.5±0.5	11±0.1	8.5±0.5
	12 mg/microliter	16.5±0.5	13±0.1	8±0.1	14±0.2	12±0.0
	18 mg/microliter	19±0.0	15±0.0	15±0.0	16±0.2	14±0.1
C. albicans	6 mg/microliter	15±0.1	13±0.2	10±0.1	11±0.1	8±0.1
	12 mg/microliter	19.5±0.5	14±0.0	14±0.0	16±0.1	10.5±0.5
	18 mg/microliter	22±0.0	14.5±0.5	14.5±0.5	19±0.0	12±0.1
Rhizopus sp.	6 mg/microliter	12±0.1	13±0.1	9.5±0.5	11.5±0.0	14±0.1
	12 mg/microliter	18±0.0	14±0.1	13±0.1	17.5±0.5	18±0.5-0
	18 mg/microliter	20±0.1	16±0.1	16±0.0	19±0.1	22±0.1
Aspergillus sp.	6 mg/microliter	13±0.0	11±0.0	10±0.2	14±0.1	14±0.1
	12 mg/microliter	15±0.2	17±0.0	15±0.0	15±0.0	16±0.0
	18 mg/microliter	18±0.1	21±0.0	21±0.0	16±0.0	20±0.0

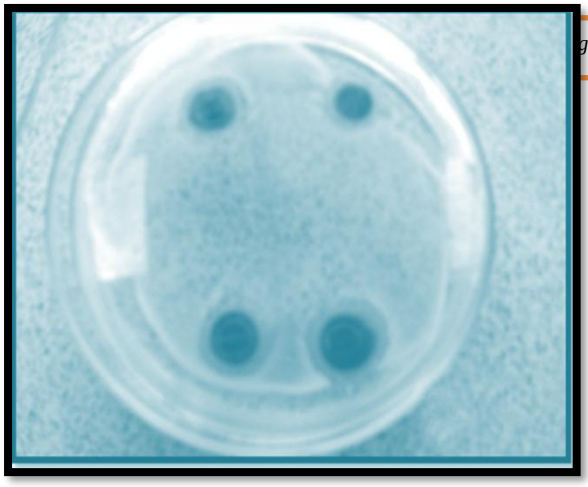


Fig 2. Image of Ethanolic extract of *Tagetes minuta* against *E. coli*

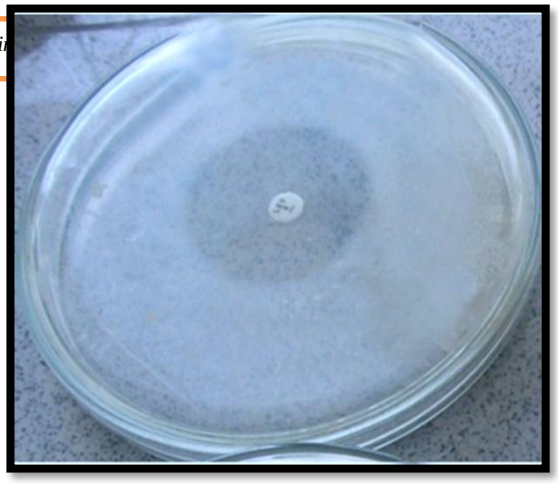
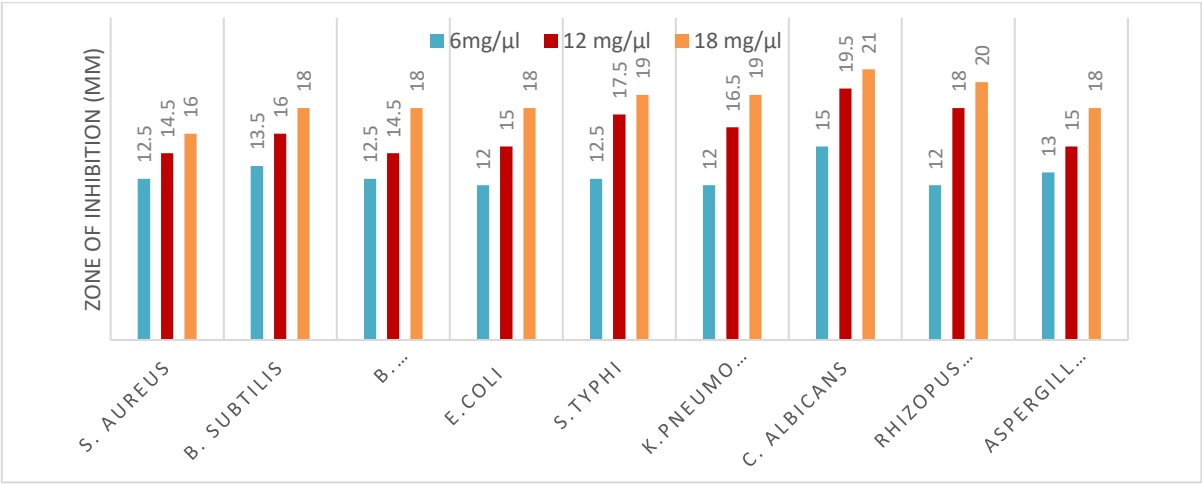
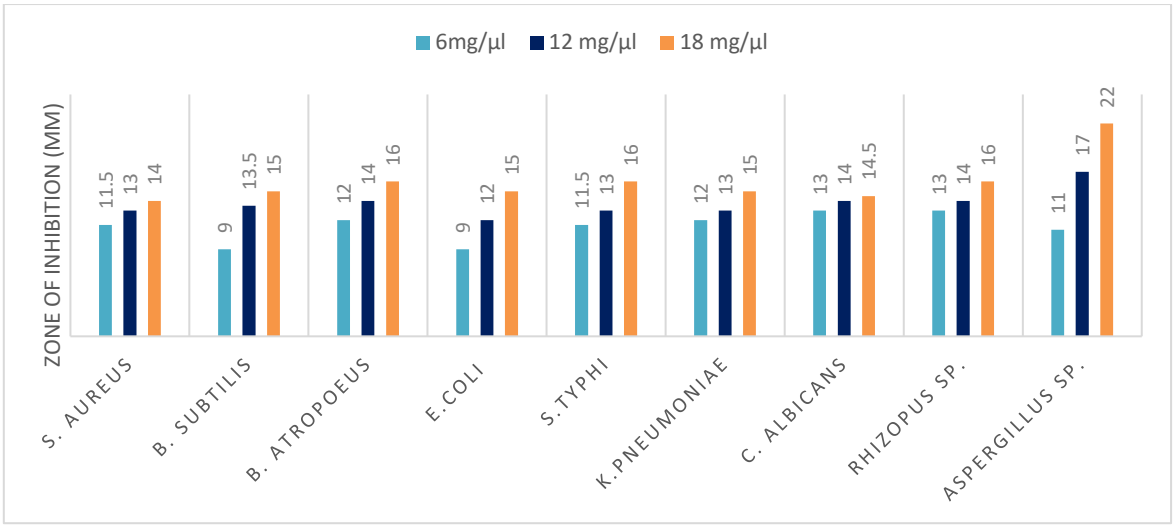


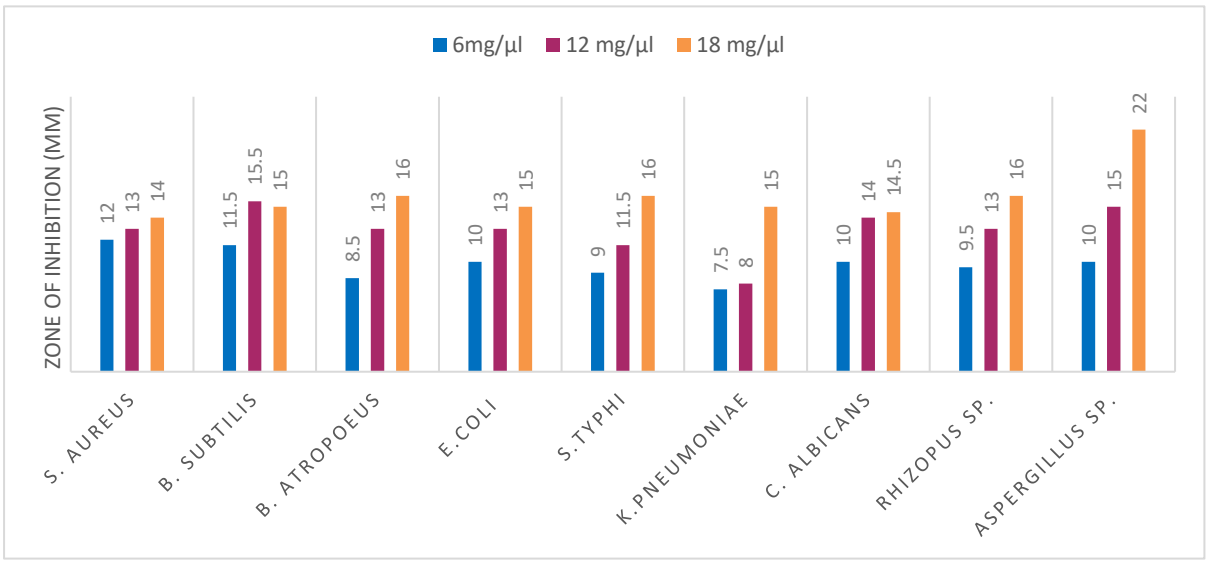
Fig 3. Image of Azithromycin (standard) against *E. coli*



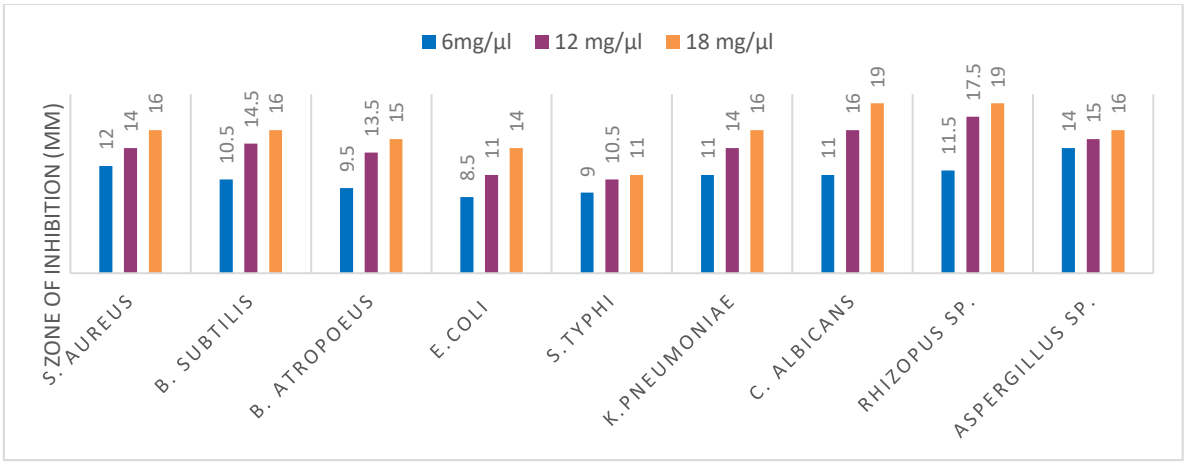
Graph.1. Antimicrobial activity of the ethan Oxalis corniculata L olic extract of *Tagetes minuta*



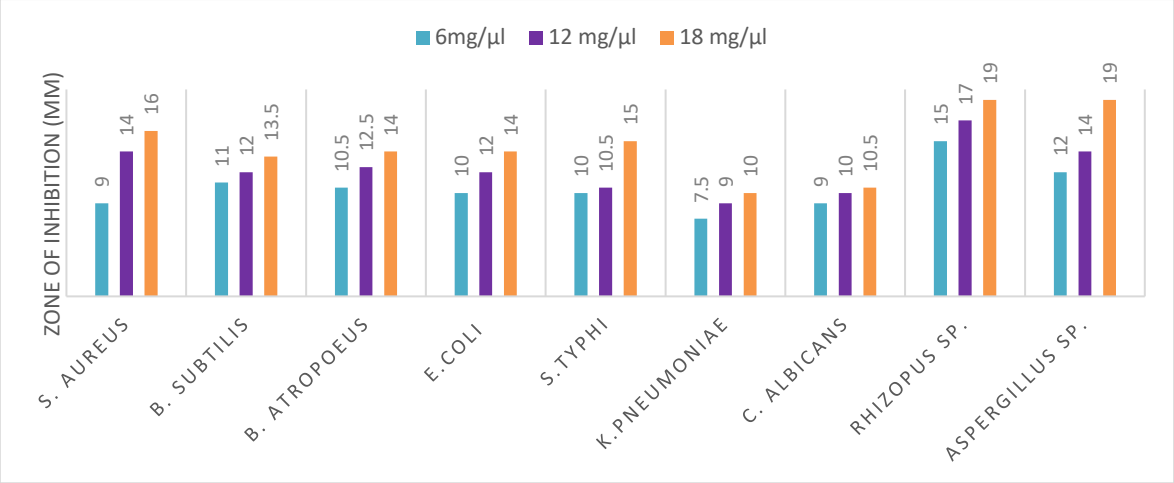
Graph.2. Antimicrobial activity of the Methanolic extract of *Tagetes minuta*



Graph.3. Antimicrobial activity of the ethyl acetate extract of *Tagetes minuta*



Graph.4. Antimicrobial activity of the hexane extract of *Tagetes minuta*



Graph.5. Antimicrobial activity of the aqueous extract of *Tagetes minuta*

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Recommendation

The present study recommends additional in-vitro and in-vivo studies and clinical trials to improve novel antimicrobial agents in this era of antimicrobial resistance.

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Conflict of Interest

The authors declare that they have no potential competing interest.

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