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Analysis of Amaranthus spinosus Extract In Vitro Combination Activity With Antibiotics On Their Antibacterial Efficacy Against Multi-Drug Resistant Streptococcus pyogenes

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ABSTRACT
The purpose of this study was to evaluate the antibacterial efficacy of Amaranthus spinosus methanol extract in combination with standard antibiotics against multi-drug resistant Streptococcus pyogenes. Alkaloids, flavonoids, phenols, tannins, and saponins were all found in the methanol extract of Amaranthus spinosus (A. spinosus) after the phytochemicals were qualitatively analyzed. The colony suspension method was used to standardize Streptococcus pyogenes bacteria for the assessment of their antibiotic susceptibility. The suspension's concentration was 1.5 106 CFU/mL after being matched to 0.5 McFarland standards. Under the guidelines of the Kirby- Bauer test, an antimicrobial susceptibility test was conducted using Mueller Hinton agar and both disc and well diffusion procedures. The bacterial sample was injected into Mueller Hinton agar plates at a volume of 50 L. The results demonstrated a reduction in the zone of inhibition of A. spinosus with Methicillin (10mcg) and A. spinosus with Penicillin of -0.9 mm and -0.4 mm from the zone of inhibition of the plant extract alone, respectively. A. spinosus and Ciprofloxacin (5 mcg) together increased the diameter of their inhibitory zone by 2 0.3 mm, whereas Tetracycline (30 mcg) did so by 1 0.2 mm. To sum up, combining plant extract with ineffective antibiotics did not result in positive significance, whereas combining efficient antibiotics with plant extract resulted in an expansion of the antibiotics' zone of inhibition, which showed positive significant activity. When applied to additional pharmacological investigations, this may be significant in improving the effectiveness of antibiotics against a variety of multi-drug resistant microbes and may hold promising therapeutic components.

Keywords: Combination; phytochemicals; MDR Streptococcus pyogenes; antibiotics; Amaranthus spinosus

I. INTRODUCTION

Gram-positive, facultative extracellular bacteria make up Streptococcus pyogenes. The bacteria have the morphology of coccus. Skin and respiratory infections are just two of the many diverse infections they produce. They cause illnesses such post-streptococcal, glomerulonephritis, necrotizing fasciitis, rheumatic and scarlet fever, impetigo, and necrotizing fasciitis [1]. The illnesses have been recognized throughout history for more than 2,000 years and are still a risk to world health [2]. Low-income nations continue to bear the burden of this dangerous virus, which raises the mortality rate year-round [3].

Several infections in people are brought on by the human-adapted bacterium Streptococcus pyogenes. Streptococcal throat infection and streptococcal toxic shock syndrome infection, respectively, are two examples of the wide range of illnesses they can cause [4]. The Streptococcus pyogenes' virulence factors, regulators, and regulatory networks all play a role in infection production [5]. In order to infect their host cells and tissues, the bacteria subject themselves to adhesion, colonization, and the formation of biofilm and microcolonies [6].

S. pyogenes' ability to invade enables colonization at the location where they manage to avoid phagocytosis. The host cells and tissues are then destroyed by a number of poisons released by them [7]. Streptococcus pyogenes carriers don't exhibit any symptoms. There are no symptoms associated with the organism's proven presence in their posterior pharynx [8].

Teichoic acid, protein M peptidoglycan, and other sophisticated virulence factors are expressed by multi-drug resistant Streptococcus pyogenes and may interfere with their normal functions [9]. Their virulence

factors are closely related to the bacteria's capacity to modify their metabolism in order to survive in a variety of unfavorable settings and hosts [10]. The fact that the bacteria are the source of infections at nearly all of their host's sites suggests that they have evolved to live in physiologically different anatomical places [11].

Multi-Drug Resistant *Streptococcus pyogenes*

MDR Complex virulence pathways are present in Streptococcus pyogenes. They change genetically over time, giving rise to several strains with different infections [12]. They have the capacity to experience mutations in the gene encoding the antibiotic binding protein. Beta-lactam antibiotics and other kinds of antibiotics are eventually no longer effective against the bacteria [13]. The proliferation of S. pyogenes that is resistant to antibiotics has caused the resistant strains to spread to other bacterial strains [14]. Since the previous decade, S. pyogenes strains have become the main source of invasive infections worldwide. Compared to the previous low toxin producers, they have become more toxic [15]. Genetic manipulations or phenotypic observations can be used to experiment with the altered bacterial gene sequence [16].

Clones with mutations spread from one bacterium to another. Bacteria serotype-related genes indicate that T-antigens, M-proteins, and by-products of other associated genes are direct indicators of their propensity for infection at a given location [17]. When latent bacteria come into contact with antibiotic-resistant strains, they mutate. They change into resistant, aggressive forms [18]. Their ways of virulence have been influenced by this evolutionary transition and genetic modification [19]. To limit the spread of these resistant phenotypic bacterial strains, various preventive actions must be followed [20].

For developing penicillin, Fleming was awarded a noble prize in 1945. On the use of antibiotics and their developing mechanisms, he foresaw and informed. The effectiveness of beta-lactam medicines against certain bacteria has decreased.

***Amaranthus spinosus* (*A. spinosus*) as aPotentialPlant in Therapy**

Amaranthus spinosus is a member of the Amaranthaceae family. In Hindi, the plant is known as cauleyi or kanteli and is also referred to as "pig weed" or "spiny amaranth" [22]. India is where it is grown nationwide [23]. A. spinosus has a wide range of medicinal uses, including those for pain relief, diabetes prevention, spasmolysis, anti-inflammatory treatment, bronchodilation, spermatogenesis, hepatoprotection, leprosy treatment, infertility treatment, antitumor treatment, antimalarial treatment, and leucorrhea [24].

Amaranthus spinosus has demonstrated a broad spectrum of antibacterial activity that can be used as a distinctive antimicrobial agent triggering its potential treatment [25]. The grains, fragile stems, shoots, leaves, and leaves all have nutritional value. Since then, boiled roots and leaves have been used traditionally to treat a variety of illnesses [26]. As a natural treatment for many ailments, it consequently has exceptional potential for success[27].

The plant contains a wide variety of phytochemicals, including carotenoids, alkaloids, phenolic acids, saponin, tannins, steroids, terpenoids, flavonoids, and amino acids [28]. Additionally, they contain dietary elements of lipids, fats, proteins, fiber, and a number of minerals. These substances are crucial for therapy [29]. In order to increase the efficacy of the antibiotics being used in suppressing a wide spectrum of resistant microbes, it may be more important to combine plant phytochemicals with antibiotics [30].

II. MATERIALS AND METHODS

Collection of Plant Material

Amaranthus spinosus plant leaves in good health were collected in April 2020 from a field at the Glocal University campus Nursery in Saharanpur, India. To remove dirt and dust, the leaves were washed under flowing water from the faucet. The leaves were then minced in a blender after being shade-dried for 20 days. For further analysis, minced samples were placed in a sterilized container that was then carefully closed with its cap and maintained in a dark location. The Botany department at Glocal University in India was given credit for authentication.

Plant Extraction Using the Methanol Solvent

Amaranthus spinosus (100 g) dried, powdered leaves were extracted with methanol. The majority of polar and semi-polar phytochemicals can be extracted since methanol has a strong polarity index of 10.2. Soxhlet extractor equipment was used for the extraction process. On a sheet of filter paper, 10 grams of the minced plant sample were placed, folded, and put inside the thimble portion of the instrument. The temperature was raised to 64.7 °C, the boiling point of methanol. For eight hours, the extraction process was performed repeatedly until the solvent in the thimble was colorless. Using Whatman No. 1, the extracted sample was filtered. A rotating evaporator set at each solvent's boiling point was used to evaporate the filter paper and other materials. The extracts were chilled for subsequent use and dried in a vacuum oven at 37.0°C.

The Qualitative Analysis on Phytochemicals Present in *Amaranthus spinosus* Methanol Extract

Amaranthus spinosus extract was qualitatively analyzed for the presence of; alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, and saponins.

The Test for Alkaloids Dragendorff's reagents test

2 ml of *Amaranthus spinosus* methanol extract poured into the test tube followed by few drops of Dragendorff's reagent. The formation of a reddish-brown precipitate was to indicate the presence of alkaloids.

Test Phenols

Lead acetate test: 2 ml of *Amaranthus spinosus* methanol extract in the test tube, 3 drops of 1% ferric chloride were added. The observation of red-brown color was to indicate the presence of phenols.

Test for Tannins

Ferric chloride test: 2 ml of *Amaranthus spinosus* methanol extract in the test tube, a few drops of 5% ferric chloride added. Blue-violet precipitation wasto indicate the presence of tannins.

Test for Saponins

Foam test: 10 ml of *Amaranthus spinosus* methanol extract in the test tube vigorously was shaken for a minute. The formation of foam was to indicate the presence of saponins.

Test for Flavonoids

Sodium Hydroxide Test: To 2 ml of *Amaranthus spinosus* methanol extract in the test tube, additional 20% sodium hydroxides were added,followed by drops of hydrochloric acid. The formation of yellow precipitate was to indicate the presence of flavonoids.

Test for Steroids and Terpenoids

Salkowski test: To 2 ml of *Amaranthus spinosus* methanol extract in the test tube, few drops of chloroform and sulphuric acid added. The reddish-brown precipitate was to indicate the presence of steroids. For the test for terpenoids, 1ml of chloroform added; the formation of chloroform layer and change of color to greenish fluorescence indicates the presence of terpenoids in *Amaranthusspinosus* methanol extract.

Multidrug-Resistant *Streptococcus pyogenes*

Bacteria Samples

The bacteria were obtained from IMTECH Chandigarh.

Identification of the Bacteria

Streptococcus pyogenes bacteria were identified by growing on blood agar plates. The samplesstreaked on the media, incubated at 37°C for 24 hours. There was an observation of beta- hemolytic, dome-shaped smooth colonies with clear margins.

Media Used

Mueller Hinton agar.

Preparation of Mueller Hinton agar Media

38 grams of Mueller Hinton agar powder added to one liter of distilled water. It was stirred to dissolve. The sterilization was done by autoclave at 121°C for 15 minutes. The liquid media was poured into the Petri dish and left to stand for 30 minutes to allow the media to solidify.

Antibiotics Used

The susceptibility test discs of Methicillin (10mcg), Penicillin –G (10 mcg), Ciprofloxacin (5mcg) and, tetracycline (30 mcg) were purchased from Himedia company.

Antibiotics Susceptibility Testing

The *Streptococcus pyogenes* strain was standardized using the colony suspension method. The suspension was then matched with 0.5 McFarland standards ensuing resultant concentration of 1.5×10^6 CFU/mL [31]. Both diskand well agar diffusion methods were used for theantibacterial susceptibility test [32]. The Mueller Hinton agar plates were inoculated with 50 µL of the bacterial sample and spread on the surface usinga sterilized glass spread. The plates were allowed tostand for half an hour. The sterilized 6 mm cork borer, was used to bore triple wells into each plate. Each of the wells was loaded with 100 µL *Amaranthus spinosus* methanolextract alone. The antibacterial susceptibility test with antibiotics was done by disc agar diffusion method, and the combination of *Amaranthus spinosus* methanol andantibiotics on well agar diffusion. All the three categorized plates wereallowed to settle for half anhour then later placed into the incubator. The plateswere incubated at 37°C for 24 hours. Thereafter diameter zones of inhibitions were determined fromthe three plates and interpreted using zone of inhibition diameter interpretative standards [33].

III. RESULTS AND DISCUSSION

The preparation for the stock solutions for phytochemical analysis; was done by dissolving 1mg of the dried *Amaranthus spinosus* methanol extract sample into 10 ml of methanol solvent [34].The dissolved solution was used in preliminary qualitative analysis for the phytochemicals identification.

The qualitative analysis of phytochemicals presents in *Amaranthus spinosus* methanol extract revealed the presence of alkaloids, flavonoids, phenols, tannins, and saponins. Terpenoids and steroids were not qualitatively identified (Fig. 1 and, Table 1).

Dragendorff's reagent test led to the formation of a reddish-brown precipitate which indicated the presence of alkaloids. The lead acetate test led to the formation of red-brown color. Phenols were present. The Ferric chloride test led to blue-violet precipitation; revealed the presence of tannins. The foam test led to the formation of foam after vigorously shaken indicated the presence of saponins. Sodium hydroxides test led to the formation of a yellow precipitate which indicated the presence of flavonoids.

Table 1. Qualitative analysis on phytochemicals present in *Amaranthus Spinosus* methanol extract

S.no	Phytochemicals	*Amaranthus spinosus*
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Phenols	+
5	Tannins	+
6	Steroids	-
7	Terpenoids	-

+ [Presence]; - [Absence]

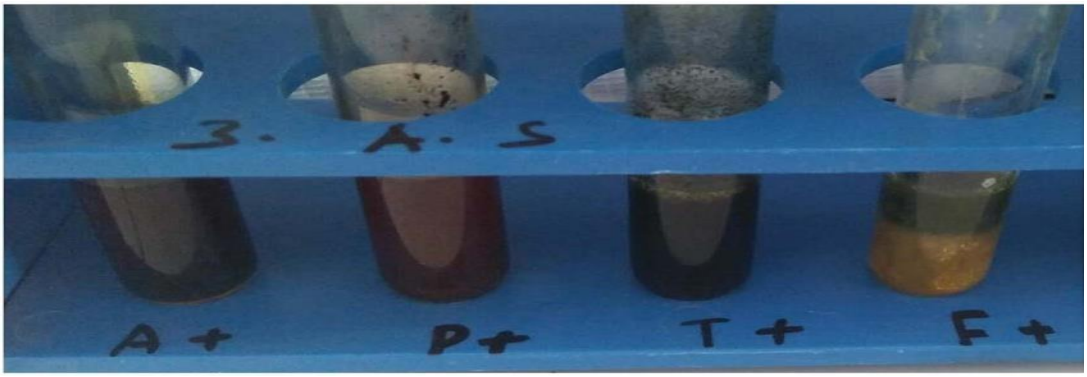

Fig. 1. Qualitative analysis on phytochemicals present in *Amaranthus spinosus* methanol extract
Presence of alkaloids (A+), phenols (P+), flavonoides (F+), Tannins (TA+)

Steroids and terpenoids; were qualitatively tested by the Salkowski test. There was no observation of reddish-brown precipitate to reveal steroids presence and no chloroform layer formed, no change of color to greenish fluorescence observed to reveal the presence of terpenoids.The diameter zone of inhibition on MDR *Streptococcus pyogenes* with *A. spinosus* methanol extract alone was 23 ± 0.9 mm. In Methicillin (10 mcg) was 08 ± 0.5 mm. Penicilli –G (10 mcg) 04 ± 0.5 mm. Ciprofloxacin (5mcg) 27 ± 0.5 mm. Tetracycline (30 mcg) 24 ± 0.5 mm. While their combination of the extract with Methicillin (10mcg) was 23 ±0.7 mm, *A. spinosus* with Penicillin –G (10 mcg) 22 ±0.5 mm, *A. spinosus* with Ciprofloxacin (5mcg) 29 ±0.8 mm and *A. spinosus* with Tetracycline (30 mcg) 25 ±0.7 mm (Table 2).

Table 2. Antibacterial activity of *Amaranthus spinosus* methanol extract and their combination with antibiotics against multi-drug resistant (MDR) *Streptococcus pyogenes*

S.no	Anti-bacterial agent against MRSA	Diameter of zone of inhibition (mm) ±*SD*
Methanol Plant extract (100 µl)		
1	*Amaranthus spinosus*	23 ± 0.9
Antibiotics		
2	Methicillin (10mcg)	08 ± 0.5
	Penicillin-G (10 units)	04 ± 0.5
	Ciprofloxacin (5 mcg)	27 ± 0.5
	Tetracycline (30 mcg)	24 ± 0.5
Combination(100µl)		
3	*A. spinosus*+ Methicillin (10mcg)	23 ±0.7
	A. spinosus+ Penicillin-G (10 units)	22 ±0.5
	A. spinosus+ Ciprofloxacin (5 mcg)	29 ±0.8
	A. spinosus+ Tetracycline (30 mcg)	25 ±0.7

+ [Combined]; ± **[**Standard deviation]

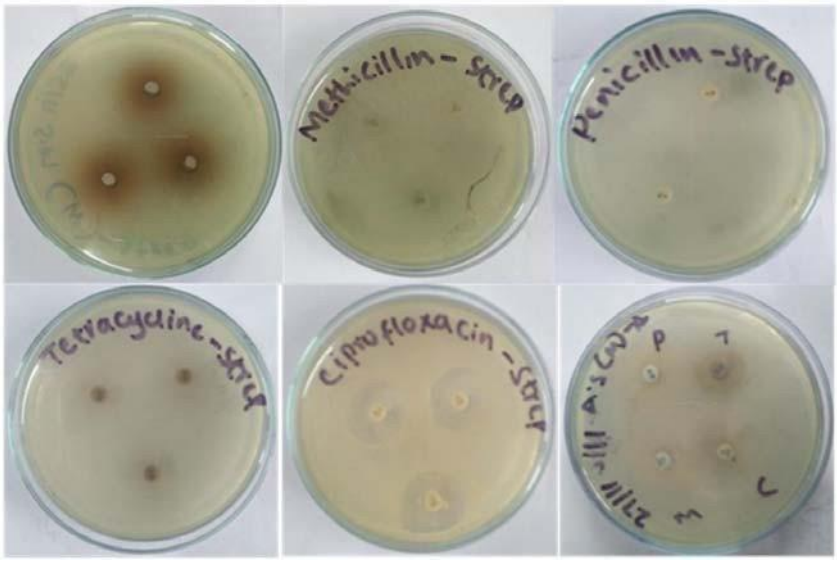

Fig. 2. The diameter zone of inhibition on *Streptococcus pyogenes*

(a) *Amaranthus spinosus* methanol extract, (b) Methicillin (10 mcg), (c) Penicillin (10 units), (d) Tetracycline (30 mcg),(c) Ciprofloxacin (5mcg), and (d) Combination of antibiotics with *Amaranthus spinosus* methanol extract

The combination of *A. spinosus* with Methicillin (10 mcg) and *A. spinosus* with Penicillin showed a decrease of - 0.9 mm and - 0.4 mm respectively from the zone of inhibition of the extract alone. Thecombination of *A. spinosus* with Ciprofloxacin(5mcg) and *A. spinosus* with Tetracycline (30 mcg) showed an increase of 2 ±0.3 mm and 1 ±0.2 mm on their diameter zone of inhibition respectively. This revealed the efficacy of the combination of *A. spinosus* methanol extractwith antibiotics against MDR *Streptococcus pyogenes*

IV. CONCLUSION

Screening for the presence of bioactive components was the primary goal of the qualitative analysis of the phytochemicals found in the methanol extract of Amaranthus spinosus. These elements are important because they have the ability to fight off bacteria that cause infections and are therefore employed in many treatments.When taken in conjunction with Ciprofloxacin (5 mcg) and Tetracycline (30 mcg) to treat MDR Streptococcus pyogenes, Amaranthus spinosus methanol extract significantly increased the diameter of the bacteria's zone of inhibition. This work will significantly advance the effectiveness of antibiotics. Antibiotics can be strengthened to fight more pathogenic germs with a larger spectrum.

REFERENCES

[1]. Delfani S, Mohammadrezaei-KhorramabadiR, Abbaszadeh S, Naghdi N, Shahsavari S. Phytotherapy for Streptococcus pyogenes. Int. J. Pharm. Sci. Res. 2017;9:513.
[2]. Ferretti JJ, Stevens DL, Fischetti VA. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016. Available:http://www.ncbi.nlm.nih.gov/books/NBK333424
[3]. Efstratiou A, Lamagni T. Epidemiology of Streptococcus pyogenes. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2017.
[4]. PMID: 26866237.
[5]. Brouwer S, Barnett TC, Rivera HernandezT, Rohde M, Walker MJ. Streptococcus pyogenes adhesion and colonization. FEBS letters. 2016;590:3739-3757.
[6]. Vega LA, Malke H, McIver KS. Virulence- related transcriptional regulators of Streptococcus pyogenes. Streptococcuspyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016.
[7]. PMID: 26866215.
[8]. Fiedler T, Köller T, Kreikemeyer B. Streptococcus pyogenes biofilms—formation, biology, and clinical relevance. Front. Cell. Infect. Microbio. 2015;5:15.
[9]. Reglinski M, Sriskandan S. Streptococcus pyogenes. In Molecular medical microbiology. 2015; DOI:10.1016/B978-0- 12-397169-2.00038-X:675-716.
[10]. Flores AR, Jewell BE, Olsen RJ, Shelburne III SA, Fittipaldi N, Beres SB, et al. Asymptomatic carriage of group A Streptococcus is associated with eliminationof capsule production. Infect. Immun. 2014;82;3958-3967.
[11]. Laabei M, Ermert D. Catch me if you can: Streptococcus pyogenes complement evasion strategies. J. Innate. Immun. 2019;11:3-12.
[12]. Pancholi V, Caparon M. Streptococcus pyogenes metabolism. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016.PMID: 26866220.
[13]. Young C, Holder RC, Dubois L, Reid SD.Streptococcus pyogenes biofilm. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016. PMID: 26866222.
[14]. Le Breton Y, Belew AT, Valdes KM, IslamE, Curry P, Tettelin H, et al. Essential genesin the core genome of the human pathogen Streptococcus pyogenes. Sci. Rep.2015;5:1-13.
[15]. Hayes A, Lacey JA, Morris JM, Davies MR,Tong SY. Restricted sequence variation in Streptococcus pyogenes penicillin binding proteins. mSphere. 2020; 5.
[16]. Musser JM, Beres SB, Zhu L, Olsen RJ, Vuopio J, Hyyryläinen HL, et al. Reduced invitro susceptibility of streptococcus pyogenes to β-lactam antibiotics associated with mutations in the pbp2x gene is geographically widespread. J. Clin. Microbiol. 2020;58.
[17]. Zhu L, Olsen RJ, Nasser W, de la Riva Morales I, Musser JM. Trading capsule for increasedcytotoxin production: contribution to virulence of a newly emerged clade of emm89 Streptococcus pyogenes. MBio. 2015;1378:15.
[18]. Maruyama F, Watanabe T, Nakagawa I. Streptococcus pyogenes genomics. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016. PMID: 26866228.
[19]. Bessen DE. Molecular basis of serotyping and the underlying genetic organization of Streptococcus pyogenes. Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]; 2016. PMID: 26866230.
[20]. Rohde M, Cleary PP. Adhesionand invasion of Streptococcus pyogenes into host cells and clinical relevance of intracellular streptococci. Streptococcuspyogenes: Basic Biology to Clinical Manifestations[Internet]; 2016. PMID: 26866223.
[21]. Wilkening RV, Federle MJ. Evolutionary constraints shaping Streptococcuspyogenes– host interactions. Trends Microbiol. 2017;25:562-572.
[22]. Barnett TC, Bowen AC, Carapetis JR. The fall and rise of Group A Streptococcus diseases. Epidemiol. Infect. 2019;147.
[23]. Hanage WP, Shelburne III SA. Streptococcus pyogenes with reduced susceptibility to β-lactams: how big an alarm bell? 2020;71:205-206.
[24]. Gotyal DM, Hiremath SK, Sarangi MS. A Review of Amaranthus Spinosus Linn: APotential Medicinal Plant. IJAMY. 2016; 9:13.
[25]. Kumar R, Jindal S, Gupta N, Rana R. An inside review of Amaranthus spinosus Linn: A potential medicinal plant of India. Int. J. Pharm. Pharm. Sci. 2014; 4:643-653.
[26]. Tanmoy G, Arijit M, Tanushree S,Jagadish S, Kumar MT. Pharmacologicalactions and phytoconstituents of Amaranthus spinosus Linn: A review. Int. J. Pharmacogn. Phytochem. Res. 2014;6:405-413.
[27]. Sheeba AM, Deepthi SR, Mini I. Evaluationof antimicrobial potential of an invasive weed Amaranthus spinosus L. In Prospects in Bioscience: Addressing the Issues. Springer, India. 2012;117-123.
[28]. Alegbejo JO. Nutritional value and utilization of Amaranthus (Amaranthus spp.)–a review. BAJOPAS. 2013;6:136-143.
[29]. Kawade RM, Ghiware NB, Sarje SK, Vadvalkar SM. A pharmacognostic and pharmacological review: Amaranthus spinosus. World. J. Pharm Sci. 2013; 2:2099-2110.
[30]. Sharma I, Kasture P, Umrao A, Rao J, Rao G. Phytochemical Analysis of Amaranthus spinosus Linn.: An in vitro Analysis. J. Emerg. Investig. 2021;2.
[31]. Ganjare A, Raut, N. Nutritional and medicinal potential of Amaranthus spinosus. J. Pharmacogn. Phytochem. 2019;8:3149-3156.
[32]. Onyancha W, Ali MI, Sharma G, Moin S. Synergistic potential of herbal plants and conventional antibiotics against multidrug-resistant bacteria. Medicinal Plants- International Journal of Phytomedicinesand Related Industries. 2021;13:13-21.
[33]. Souza FA, da Silva VG, Bitencourt TB. Use of McFarland Standards and Spectrophotometry for Yarrowia Lipolytica QU69 cell counting. Int. J. Agric. Environ. Biotechnol. 2017;5:4.
[34]. Wayne P. Performance Standards for Antimicrobial Susceptibility Testing, Ninth Informational Supplement. 2008;11.
[35]. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 1966;45:493-496.
[36]. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science and Business Media. 1998;1-36.