*International Journal of Biochemistry Research & Review*

*29(9): 101-107, 2020; Article no.IJBCRR.61962*
*ISSN: 2231-086X, NLM ID: 101654445*

# Characterization and Antibacterial Potential of *Tithonia diversifolia* Extract and Its Iron II Nanoparticles

**Hellen Chebet[1*], Johnson Kinyua[1], Patrick Kareru[2] and Njiema Gitaari[2]**

*[1]Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200, Nairobi, Kenya.*
*[2]Department of Chemistry, College of Pure and Applied Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000-00200, Nairobi, Kenya.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author HC designed the study, carried out laboratory experiments, wrote the protocol and wrote the first draft of the manuscript. Author PK guided on instrumentation and reagents provision. Author JK managed literature searches. Author NG managed the analyses of the study. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IJBCRR/2020/v29i930229
Editor(s):
(1) Prof. Cheorl-Ho Kim, Sungkyunkwan University, South Korea.
Reviewers:
(1) Priyawan Rachmadi, Airlangga University, Indonesia.
(2) Najma Ismail, Aliah University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61962

***Original Research Article***

***Received 10 August 2020***
***Accepted 15 October 2020***
***Published 02 December 2020***

## ABSTRACT

Several plants worldwide have exhibited potentiality in human pathogen eradication. The aim of this work was to characterize the compounds and determine antibacterial efficiency of *Tithonia diversifolia* essential oils and its iron II nanoparticles. The experimental test was done against gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). Characterization of the compounds was done using UV-VIS and FTIR to determine the functional groups that are present. Antibacterial activity was done using disc diffusion method by Beer Lambert. The results obtained indicated that the crude extract at 0.1% v/v concentration was effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* only, while the complexed sample was significantly effective against all microbes under test at the corresponding concentration. The outcome showed comparable results to the positive controls used and therefore, the plant extract was effective against human disease causing microbes and is recommendable for use against them.

**Corresponding author: E-mail: helcheb26@gmail.com;*

*Keywords: Extract; iron II nanoparticles; bacteria; antibacterial activity.*

## 1. INTRODUCTION

As studied earlier, higher plants are believed to be good source of medicine with no side effect, this has led to improvement of the lives both in urban and rural setup. In the early years, plants were used by instinct to combat different ailments and it truly worked [1]. There has been an increase in interest by scientist to know about the compounds that are in these plants since they induce therapeutic effect [2]. Many of the therapeutic plants were discovered in the early 18$^{th}$ century and their use in animals and humans has shown larger extent in treatment [3]. Some were discovered to be toxic to human and hence eliminated from the list of medicinal plants. The first medicinal plant was studied in 19$^{th}$ century and its compounds characterized. In the recent times, plant efficiency and effectiveness has been studied by use of advanced machines, it led to an increment in extraction of essential elements in plants that are of medicinal importance to human and animal system [4,5]. It has been proven that many plants are good antimicrobial and antioxidants, there are also some that are anti-cancer [6,7]. There is also an aspect on nanotechnology whereby, use of nanoparticles (NPs) is increasing in both scientific research and industrial applications due to a wide variety of potential applications in biomedical, optical and electronic fields [8,9]. Nanoparticles have been used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions [10,11] and treatments of various diseases. It has been found that Synthesis of nanoparticles is a valuable approach in green nanotechnology [2]. Biological resources such as bacteria, algae, fungi and plants have been used for the synthesis of low-cost, energy-efficient, and nontoxic environmental friendly metallic nanoparticles of both metal and metal oxide NPs [12]. In this study, iron nanoparticles will be generated to examine its effectiveness in combating pathogens compared to the crude extracts [13,14].

The tithonia vegetation is found to be containing high proteins, high fibre contents, it is high in nutrients during vegetative stage as compared to the flowering stage. The foliage has high mineral content of about 16% dry matter and it is particularly calcium.

In Kenya, the plant has so many uses and across the nation, it is referred to as a bitter plant because of its bitter taste. It is used as an organic fertilizer in tea and compost farming [6]. Medicinal uses includes cure for constipation, sore throats, stomach pains, liver pains, indigestion and diarrhea [15]. It has been identified as a potential plant against insects such as infestation of termites in farms.

In Nigeria, it is found to be effective in treatment of diabetes mellitus, wounds and menstrual pains. In Mexico where it originates, the additional uses includes treatment of bone marrow, bruises, sprains, malaria, muscular cramps and hematomas [3]. It is used as an anti-inflammatory, treatment of skin diseases, hepatitis, cystitis, jaundice and night sweats.

The plant organs i.e. the roots, leaves and the stem contains substances nonnutritive and nutritive substances that are applicable in pharmaceutical purposes [10]. The medically active components make it applicable as discussed above. The phytochemicals including the terpenoids, saponins, phenols, tannins, alkaloids and avonoids are found to be abundant in the plant [16,17,18]. They are concentrated in the leaves followed by the roots. Trace metals are also present in the plant and includes zinc, copper, magnesium, nickel, phosphorous and iron. The terpenoids that are common includes sesquiterpenes which helps plant in its defense. The metabolite is effective against bacteria, virus, fungi and bacteria. It plays a role as anti-cancer and anti-inflammatory.

Study shows that the leaves flowers and stem contains the pinene, germacrene D, caryophyllene, cineole and bicyclogermacrene [18]. The oils vary from one country to another and this is linked to the effect of the environment to it [4]. The geographical distribution is also seen to be affected by the climate and hence growth pattern varies across the continents. The essential oils are commonly used in fragrance and in food stuffs as flavor. Its effectiveness against microbes have not been fully exploited hence this study worked to determine the potentiality [12,19,20]. Stability of the essential oils was also seen to be very low, the shelf life was short and could not be kept for longer period of time, and this led to the study's aim to complex the extract with iron II salt.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Extraction

The leaves of *Tithonia diversifolia* plant were collected from Jomo Kenyatta University of Agriculture and Technology botanical gardens, Kenya. The leaves were washed with running water and sliced to small pieces then air dried at room temperature for a week. It was then ground with an in-house blender. Clevenger apparatus was used to extract through hydro-distillation technique, extraction was done using n-hexane, ethylacetate and methanol so to capture all the essential oils with differing polarity. The solvents were removed by rotary evaporator and the collected essential oils kept at 4°c in vials.

Synthesis of iron II nanoparticles was done by addition of 0.01M ferrous chloride and the *Tithonia diversifolia* extract in ratio of 2:5. The mixture as heated in a magnetic hot stirrer at 60°c for two hours to allow reaction to take place. A colour change from pale green to brown indicated oxidation of the metal ions. pH was kept constant at 8 since it's the major influence to the reaction. The solution was centrifuged and the pellets washed with distilled water.

### 2.2 Antibacterial Activity

The microbes used were: gram positive bacteria (*Bacillus subtillis*, *Staphylococcus aureus* ATCC 25923) and gram negative bacteria (*Escherichia coli* ATCC 25992, *Pseudomonas aeruginosa* ATCC 27853). Muller Hinton nutrient agar was used to culture the bacteria. The culture medium was seeded in aseptic condition using sterilized platinum loop then incubation done.

Antibacterial effectiveness was done using disk diffusion technique. Concentrations of both the crude extract and the nanoparticles were prepared separately as follows; 0.1, 0.2, 0.5, 1.0, 2.0, 5.0%v/v by dissolving in DMSO. They were sterilized using Millipore filter and loaded over sterilized filter paper disc (7 mm diameter) according to the requisite amount, the prepared agar was introduced into sterile petri dishes and seeded with the pathogenic strains, the filter paper disc that were loaded with the different concentrations of the extract were introduced into the petri dishes containing the agar. This was done in triplicates for both the crude extract and the nanoparticles. The plates were then placed in a fridge at 5°C for 2 hours then transferred to an incubator (37°C) for 24 hours. The zone of growth inhibition was measured by vernier caliper and recorded.

The antibacterial effect of the extracts were determined in comparison with the positive controls (microcapsules for antibiograms); norfloxacin(NX), ofloxacin (OF), ceftriaxone (CTR), sulphamethoxazole (SX), amoxylclar (AMC), nitrofuractoin (NIT), nalidixic acid (NA), and gentamycin (GEN).

## 3. RESULTS AND DISCUSSION

### 3.1 Antibacterial Analysis

The zone of inhibition of *Tithonia diversifolia* crude extract is shown in Fig. 1 while Fig. 2 shows the inhibition zone exhibited by the complexed extract.

At a concentration of 5%v/v the zone of inhibition diameter against *Escherichia coli* was 16± 1.0 mm compared to inhibition zone in uncomplexed extract which was 13±1.0 mm. Similar trends were observed whereby the nanoparticles at the applied concentrations are more effective than the crude extract.

Nitrofuractoin showed a lower effectiveness on *E. coli* compared to both the extract and the nanoparticle at a concentration of 5%v/v. Test sample had higher effect on *Staphylococcus aureus* at the concentrations of 2% and 5%v/v compared to nitrofuractoin and amoxylclar standards. Inhibition of *pseudomonas aeruginosa* was higher at 1%, 2% and 5% v/v concentrations of the complex compared to inhibition by sulphamethoxazole. *Bacillus subtilis* inhibition by the extract was slightly higher at 5% concentration compared to sulphamethoxazole and highly inhibited by the nanoparticles at the same concentration than sulphamethoxazole and ceftriaxone.

The antimicrobial activity of *Tithonia diversifolia* findings were similar to those found by other researchers, however, the complexed essential oils were found to be more effective against the bacteria compared to crude and also stabilizes the compound.

### 3.2 Characterization Using UV-VIS

Samples were measured on Shimadzu UV-VIS 530A spectrophotometer for absorption. Fig. 3 shows the absorption spectra of the essential oils obtained from *Tithonia diversifolia* extract. The spectra were from the pure extract and the

complexed, measured in the wavelength range 800-200 nm. There is enhancement of peak intensities at 420 nm and 670 nm and disappearance at 320 nm for the complexed.

*Tithonia diversifolia* extract gave highest zone of inhibition against all microbes at a concentration of 5%v/v, the lowest effective concentration was at 0.1%v/v against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The effective concentrations against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were seen at 0.2%v/v, *Escherichia coli* inhibitions lowest concentration was seen at 0.5% v/v. The extract was able to inhibit all the bacteria under test at 0.5%v/v.

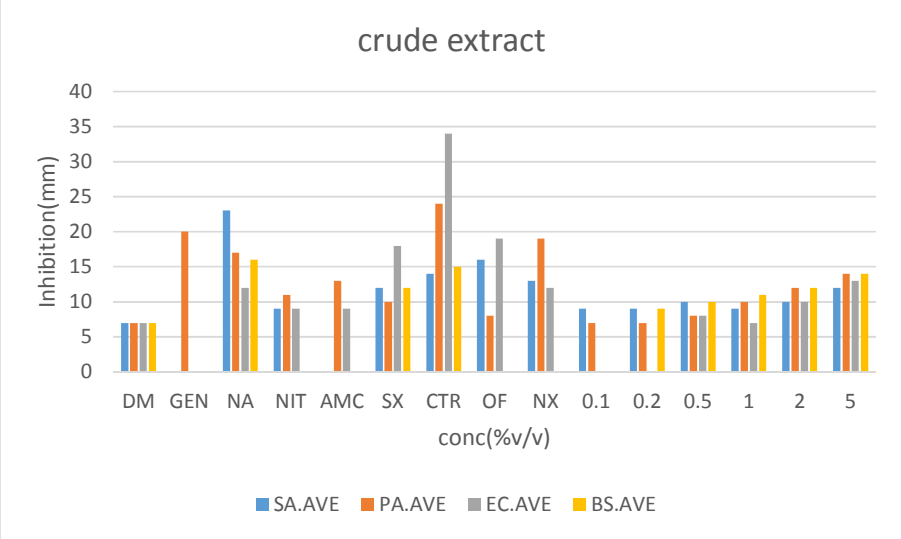


**Fig. 1. Antimicrobial activity of *Tithonia diversifolia* essential oils**
*Key: SA; Staphylococcus aureus, PA; Pseudomonas aeruginosa, EC; Escherichia coli, BS; Bacillus subtilis, DM; Dimethylsulphoxide, GEN; Gentamicin, NA; Nalidixic acid, NIT; Nitrofuractoin, AMC; Amoxylclar, SX; sulphamethoxazole, CTR; Ceftriaxone, OF; Ofloxacin, NX; Norfloxacin*

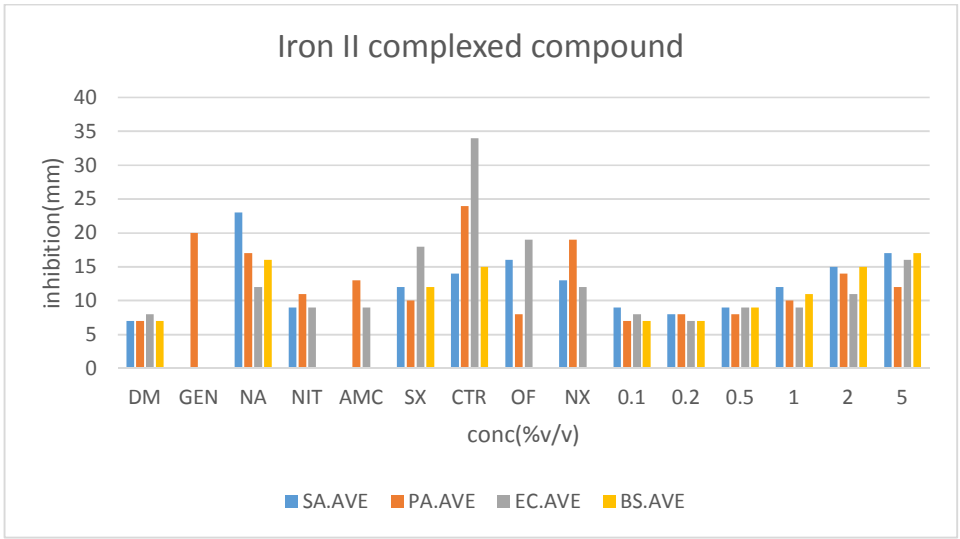


**Fig. 2. Shows the antimicrobial activity of the Iron II complex of *Tithonia diversifolia* extract**
*Key: SA; Staphylococcus aureus, PA; Pseudomonas aeruginosa, EC; Escherichia coli, BS; Bacillus subtilis, DM; Dimethylsulphoxide, GEN; Gentamicin, NA; NALIDIXIC acid, NIT; Nitrofuractoin, AMC; Amoxylclar, SX; Sulphamethoxazole, CTR; Ceftriaxone, OF; Ofloxacin, NX; Norfloxacin*

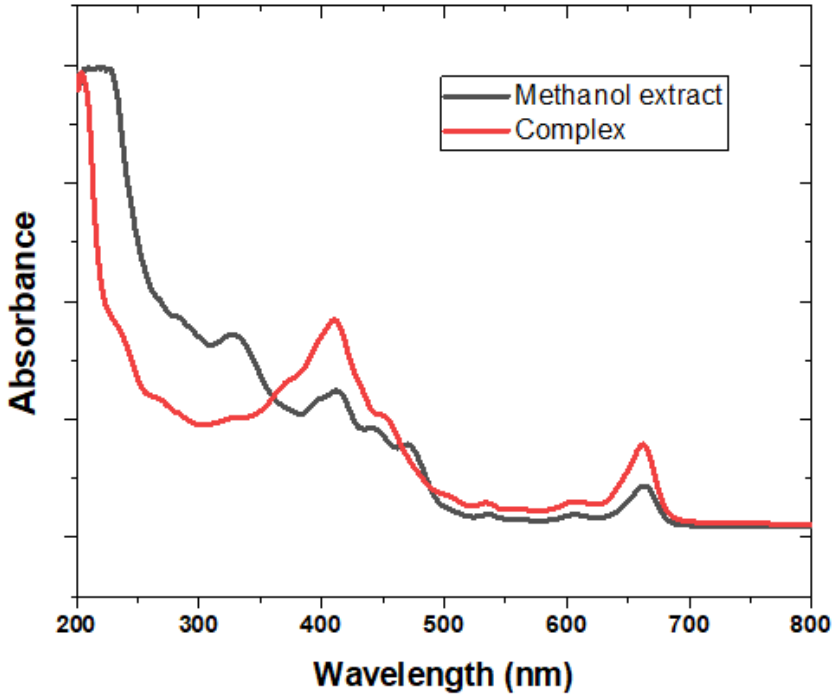


**Fig. 3. UV-VIS peaks**

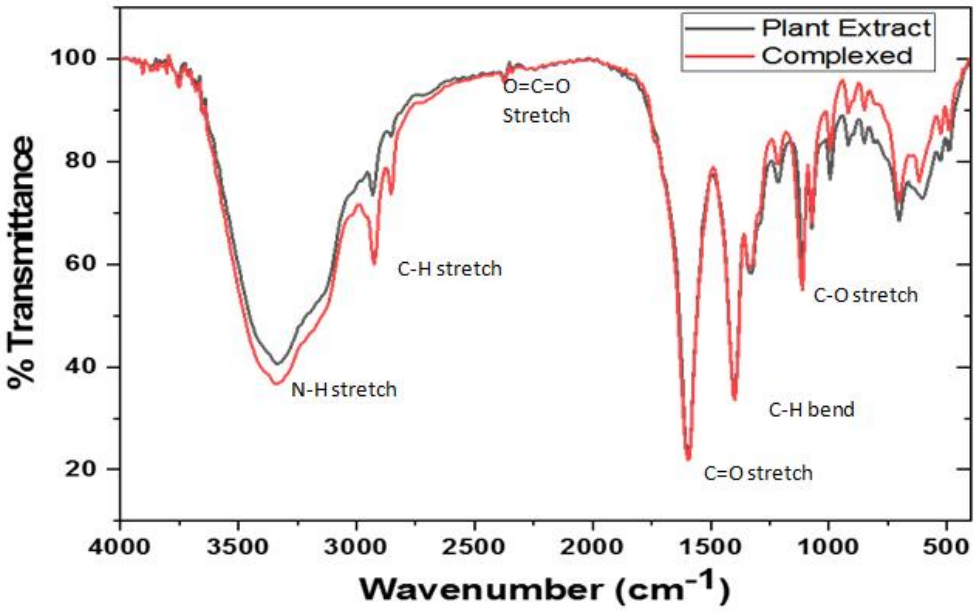


**Fig. 4. FTIR spectra of *Tithonia diversifolia* extract and its nanoparticals**

The observed absorption wavelength of the complexed extract indicated a shift towards the shorter wavelength. This shifting is attributed to the reduction of conjugation as a result of the reaction between the metal ions and the lone pairs and pi electrons in the compounds present in the extract. The size reduction of the semiconductor crystals accompanied with band

gap widening which requires higher absorbed quantum energy to excite electrons from the valence band into the conduction band.

### 3.3 Characterization Using the FTIR

Perkin-Elmer spectrometer was used to determine the functional groups in the plant. The sample to be tested was mixed with KBr to for elasticity and light penetration. Sample disc was made thin by pressing with preparing machine and placed in the FTIR for analysis.

Fig. 4 represents the absorption spectra of the essential oils from *Tithonia diversifolia* extract and the nanoparticle. The wavelength range was 500-4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Region below 1000 $cm^{-1}$ (fingerprint) of the IR spectrum contain absorption bands that characterize the entire molecule structure by vibrations of the spectrum: Deformation, combining, harmonic bands that cannot generally be attributed to normal vibrations.

The peaks indicated the functional groups that are present in the plant. Some of the bands observed in the complexed spectrum were different in intensity compared to those of the uncomplexed one. N-H, C-H and C-O groups were at a slightly different locations from those of uncomplexed extract. The shifting of the peaks for complexed extract is attributed to the formation of iron II nanoparticles by the carbonyl group.

## 4. CONCLUSION

Uncomplexed extract exhibited no inhibition to bacteria *Escherichia coli* and *Bacillus subtilis* at a concentration of 0.1%v/v unlike the complexed which inhibited the growth of all the bacteria under test at the corresponding concentration. At 5%v/v concentration, the nanoparticles inhibition of *Escherichia coli* and *Bacillus subtilis* were at 16±0.1 mm and 17±0.1 mm diameters respectively. In comparison, the crude extract inhibition zone for *Escherichia coli* and *Bacillus subtilis* at 5%v/v concentration were 13±0.1 mm and 14±0.1 mm diameter respectively. This indicates that, apart from improving the shelf life of the essential oils, nanoparticles also enhances antibacterial activity to a larger extend.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Severino V. Concepts, compounds and the alternatives of antibacterials. Ashland: Delve Publishing; 2019.
2. Ogunfolakan O, Kolawole OS, Olowe AO. *In vitro* antimicrobial activity of *Tithonia diversifolia* leaf extracts on bacterial isolates from wound infections from a Nigerian hospital. Research Journal of Medical Sciences. 2010;4(5):305-308.
3. Ana Luzia Ferreira Farias, Alex Bruno Lobato Rodrigues, Rosany Lopes Martins, Érica de Menezes Rabelo, Carlos Wagner Ferreira Farias, Sheylla Susan Moreira da Silva de Almeida. Chemical characterization, antioxidant, cytotoxic and microbiological activities of the essential oil of leaf of *Tithonia diversifolia* (Hemsl) A. Gray (Asteraceae). Pharmaceuticals. 2019;12:1.
4. Mashwani ZR, Khan T, Khan MA, Nadhman A. Synthesis in plants and plant extracts of silver nanoparticles with potent antimicrobial properties: Current status and future prospects. Applied Microbiology and Biotechnology. 2015;99(23): 9923-9934.
5. Oso B, Ogunnusi T. Antibacterial activity of methanolic extracts of *Euphorbia heterophylla* and *Tithonia diversifolia* against some microorganisms. European Journal of Medicinal Plants. 2017;20(3):1-8.
6. Kerebba N, Oyedeji AO, Byamukama R, Kuria SK, Oyedeji OO. Pesticidal activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); phytochemical isolation and characterization: A review. South African Journal of Botany. 2019;121: 366-376.
7. Stepp JR. Ethnoecology and medicinal plants of the highland Maya; 2018.
8. Volova TG, Mahapatra DK, Khanna S, Haghi AK. Natural products chemistry: Biomedical and pharmaceutical phyto-chemistry; 2020.
9. Volova TG, Mahapatra DK, Khanna S, Haghi AK. Natural products chemistry: Biomedical and pharmaceutical phyto-chemistry; 2020.
10. Thangadurai D, Sangeetha J, Prasad R. Functional bionanomaterials: From biomolecules to nanoparticles. Cham: Springer International Publishing AG; 2020.

11. Anastas PT, Perosa A, Selva M. Handbook of Green Chemistry, Green Processes, Green Nanoscience; 2013.
12. Dada AO, Inyinbor AA, Idu EI, Bello OM, Oluyori AP, Adelani-Akande TA, Okunola AA, Dada O. Effect of operational parameters, characterization and antibacterial studies of green synthesis of silver nanoparticles using *Tithonia diversifolia*. Peerj. 2018;6.
13. Thi TTT, Thi LHN, Van TH, Thi TL, Thi VTH. *Tithonia diversifolia* pectin - reduced graphene oxide and its cytotoxic activity. Materials Letters. 2016;183:127-130.
14. Tran TTT, Vu TTH, Nguyen TH. Biosynthesis of silver nanoparticles using *Tithonia diversifolia* leaf extract and their antimicrobial activity. Materials Letters. 2013;105:220-223.
15. Grumezescu AM. Nanoscale fabrication, optimization, scale-up and biological aspects of pharmaceutical nano-technology. San Diego: Elsevier Science; 2017.
16. Ahmed S. *Tithonia diversifolia* (Hemsl.) A. Gray: Ethnopharmacology, biological and phytochemical investigation. Saarbrücken: LAP LAMBERT Academic Publishing; 2014.
17. International Congress of Ethno-pharmacology. In Mukherjee PK, Jadavpur University, & Society for Ethnopharmacology (India). Traditional medicine and globalization: The future of ancient systems of medicine; 2014.
18. Okereke SC, Arunsi UO, Nosiri CI, Nwadike C. Gas chromatography mass spectrometry/Fourier transform infrared (GC-MS/FTIR) spectral analyses of *Tithonia diversifolia* (Hemsl.) A. Gray leaves. Journal of Medicinal Plants Research. 2017;11(19):345-350.
19. Douglas K. Phytochemistry and anti-microbial activities of some medicinal plants; 2016.
20. Gutierrez RM, Ballada K, Patacsil M. The antibacterial property of *Tithonia diversifolia* (Wild Sunflower) extract from Baguio-Benguet areas in the Philippines, in response to exposure to vehicular traffic. Asian Journal of Microbiology, Biotechnology & Environmental Sciences. 2015;17(1):43-52.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/61962*