IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 08, Issue 7 (July. 2018), ||V (III) || PP 01-07

Photochemical Screening with Estimation of Total Flavonoid Content in *Parthenium Hysterophorus* and In Vitro Analysis of Antimicrobial Activity

Saksham Khusharia, Nikunaj Bhardwaj
Department of Biotechnology, Noida International University, Greater Noida (UP), India
Corresponding Author: Nikunaj Bhardwaj

Abstract: There is a continuous need for human society to discover new antimicrobial compounds having diverse chemical structures and innovative mechanisms of action for novel and reemerging infectious diseases. Therefore, researchers are progressively turning their consideration to traditional medicine, looking for new leads to develop improved drugs against microbial infections. Plants yield a diverse variety of bioactive molecules, making them rich source of different types of medicines. Parthenium hysterophorus is a weed plant reported to be used as remedy for a number of diseases. The leaf extracts of Parthenium hysterophorus were assessed against all the test microorganisms. An extract from the Parthenium hysterophorus leaves were screened for their phytochemical ingredients and its antimicrobial activity against clinical isolates of many microbial cultures. Phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Cardiac Glycosides, Flavonoids, Glycosides, Phenols, Proteins, Saponins, Terpenoids, Tannins and Steroids. Agar well diffusion method was used to test the antimicrobial activity of both the standard antibiotic and plant extract. The antimicrobial activity exhibited by the leaf extract has shown significant potential in inhibiting various pathogens. The objective of the present study was to evaluate the potential of these plant extracts used in traditional healthcare system, for antibacterial activity against important human pathogenic bacteria. Thus it is a reasonable approach in drug discovery to screen traditional natural products. The results provide support for the usage of this plant in making new bioactivity compounds having antimicrobial activity.

Keywords: Parthenium hysterophorus, Antimicrobial activity, Leaf extract, Antimicrobial, Antibiotics, Phytochemical screening, Well diffusion method.

Date of Submission: 28-06-2018 Date of acceptance: 13-07-2018

I. INTRODUCTION

Plants produce a different range of bioactive chemical compounds, making them an important source of various types of medicines for therapeutic aspects. Most of the drugs today are achieved from the natural sources or semi synthetic derivatives of the natural products. The use of plant extracts and the phytochemicals has a great value in therapeutic treatments. Antibacterial are used against bacteria and antifungal are used against fungi both can be consider as antibiotics. More than 80% of individuals from developed countries use folk methods for medicine, which has compounds derived from plants having medicinal value. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. For this many plants substances have been used, which are chiefly secondary metabolites. These plants substances should be investigated properly to better understand their medicinal properties. Normally all the parts of plant possess antimicrobial properties.

Normally a weed is a plant considered uninvited in a particular condition, "a plant in the wrong place". Examples commonly are plants unwanted in human-controlled settings. Parthenium hysterophorus L., which belongs to Asteraceae is a common weed distributed worldwide. It´s decoction has been used as a folk medicine for the treatment of fever, diarrhoea, neurologic disorders, urinary infections, dysentery and malaria and as emmenagogue and several other diseases. In India it was first described as altamisa, and commonly known as the carrot grass, bitter weed, star weed, white top, wild feverfew, the Scourge of India and the congress grass. The extracts of leaf have a potent role in the fertility, fecundity and behavioral response. A sesquiterpenoid known as the parthenin, is the active compound present in this plant (Kumar *et al*., 2013a).

Phytochemicals function as antioxidants and react with free radicals to combat oxidative stress. Excessive free radicals are generated in body due to unbalanced oxidants and antioxidants ratio which results into oxidative stress. Antioxidants scavenge and control the formation of free radicals thereby preventing oxidative damage to cellular components arising as a consequence of chemical reactions involving reactive

oxygen species (ROS). Literature search exposes conducting of very insufficient studies on the antimicrobial action of *Parthenium hysterophorus* (Bhuvaneswari *et al*., 2011; Manikandan *et al*., 2011; Prakash *et al*., 2012; Sanguri *et al*., 2012). The present investigation is therefore, undertaken to test the efficacy of *Parthenium hysterophorus* weeds extracts against the bacterial and fungal pathogens.

II. MATERIALS AND METHODS

Sample collection and its extraction

The leaves of *Parthenium hysterophorus procera* were collected from the barren lands of Greater Noida. The taxonomic identities of these weeds were confirmed by Dr. Pankaj Sharma, Noida Testing Laboratory, Noida. The collected leaves were examined for their pathogenic infections and healthy leaves were selected carefully. After that the leaves were washed under tap water and then by the sterile distilled water and air dried at a temperature of 38°C for 5-6 days. After that the leaves were homogenized to a fine powder and stored in air tight bottles. Different solvents (namely ethanol, methanol, acetone, aqueous, petroleum ether, distilled water, chloroform and ethyl acetate) were used for the extraction. Homogenized leaves, 10 g each, were separately soaked in conical flasks each containing 100 mL of acetone, ethanol, methanol, sterile distilled water, petroleum ether, aqueous, chloroform and ethyl acetate. All the flasks keep on the rotary shaker (200 rpm for 24 h). Each preparation was filtered and finally concentrated to dryness under vacuum (at 40°C). The dried extract thus obtained was sterilized by overnight ultra violet-irradiation, checked for sterility on nutrient agar plates and stored at 4°C in labeled sterile bottles until further use (Aneja and Sharma, 2010; Aneja *et al*., 2011). In case of *Parthenium hysterophorus* by using Methanol, Ethanol, Ethyl acetate, Acetone, Chloroform, Petroleum Ether, Hexane and Aquas Extract, respectively 100, 80, 75, 68, 55, 32, 20 and 12 % maximum antimicrobial activity was observed.

Microorganisms Source

To conduct the experimental work, a total of six microbial cultures were selected. Of the six microbial cultures, Two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal species (*Candida albicans* and *Saccharomyces cerevisiae*) were used. All the microbial cultures were procured from Noida Testing Laboratory, Noida. The bacteria were subculture on Nutrient agar, whereas, yeast were grown on Sabouraud's dextrose agar at 37°C for 24-48 h.

PHYTOCHEMICAL ANALYSIS

Phytochemicals are the chemicals substances which are extracted from plants. These chemical substances can be classified as either primary or secondary constituents, depending on their potent role in metabolism of plant. Sugars, amino acids, proteins, purines and pyrimidines of nucleic acids are some common examples of primary constituents. Secondary constituents are Alkaloids, terpenes and phenolics which protect the plants and cab be considered as disease-preventing compounds.

Procedure for the Phytochemicals Test

The preliminary phytochemical analysis was carried out on the methanol extract using standard procedures (Edeoga HO, Okwu DE and Mbaebie BO., 2005; Yadav RNS and Munin Agarwal, 2011; Trease GE, Evans WC., 1989; Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur., 2011) to identify the phytochemical constituents. The extracts of the Parthenium hysterophorus were screened for phytochemical constituents using standard chemical test with little modification (Sasi Kumar et al, 2014 and Christy Gnana Theeba P. et al, 2015).

Test for alkaloids:

Wagner's Test:

2ml of extract was treated with the wagner's reagent. Formation of reddish brown precipitate specifies the presence of alkaloids in extract of leaves of *Parthenium hysterophorus*.

Test for flavonoids:

NaOH Test:

2ml of extract was treated with limited drops of aqueous NaOH and HCl. Formation of yellow orange color shows the occurrence of flavonoids in extract of leaves of *Parthenium hysterophorus*.

H2SO4 Test:

Extract is treated with Concentrated H2SO4. Check the color of medium. Orange color shows the presence of flavonoids in the extract of leaves of *Parthenium hysterophorus*.

Lead Acetate Test:

Extract was treated with lead acetate and keep the medium for some time. Observe the color of medium after some time. White precipitate formation shows of the occurrence of flavonoids in extract of leaves of *Parthenium hysterophorus*.

Test for tannins:

Ferric Chloride Test:

Extract was added with alcohol, after that treated with neutral ferric chloride solution. Formation of blue/ or greenish color in the solution shows the presence of tannins in extract of leaves of *Parthenium hysterophorus*.

Test for saponins:

Foam Test:

Extract was shaken strongly with water; formation of determined foam specifies the presence of saponins in extract of leaves of *Parthenium hysterophorus*.

Test for Cardiac glycosides:

Kellar-Killani Test:

A small fraction of extract was treated with glacial acetic acid (1ml), 5% FeCl3 (1 drop) and Concentrated H2SO4. Detected the color which appears in the solution, formation of reddish brown color at intersection of the two liquid layers and upper seems bluish green shows the presence of Cardiac glycosides in extract of leaves of *Parthenium hysterophorus*.

Test for quinines:

Extract was treated with Concentrated HCl, yellow color precipitate formation specifies the presence of quinones in extract of leaves of *Parthenium hysterophorus*.

Test for carbohydrates:

Molisch's Test:

A small fraction of molisch's reagent was added to each of the portion dissolved in distilled water, followed by the addition of Concentrated H2SO4 (1ml) by the wall of the test tube. The solution was then allowable to stand for few minutes, after that diluted with distilled water (5ml). Formation of red/ or violet color at interphase of the two layers specifies the existence of carbohydrates in extract of leaves of *Parthenium hysterophorus*.

Fehling's Test:

A small fraction of extract was dissolved in distilled water and after that the solution was filtered. The filtrate was heated with fehling's solution A and B (5ml) of equal volumes. Red precipitate of cuprous oxide specifies the presence of reducing sugar in extract of leaves of *Parthenium hysterophorus*.

Test for Terpenoids:

Liebermann – Burchard Test:

A small fraction of extract was treated with chloroform, acetic anhydride and H2SO4. Formation of greenish color shows the terpenoids in extract of leaves of *Parthenium hysterophorus*.

Test for Sterols:

Liebermann – Burchard Test:

A small fraction of extract was treated with chloroform, acetic anhydride and H2SO4. Observation of pink/ or red color/ or reddish brown ring shows the presence of sterols in extract of leaves of *Parthenium hysterophorus*.

H2SO4 Test:

A small fraction of extract was treated with ethanol and H2SO4 and detected the development of violet/ or green color shows the presence of sterols in extract of leaves of *Parthenium hysterophorus*.

Test for Phenols

Liebermann Test:

A small fraction of extract was heated with NaNO3, H2SO4. Now the solution was diluted with water and then adds dilute NaOH and detected the development of deep red/ or green/ or blue color shows the presence of phenols in extract of leaves of *Parthenium hysterophorus*.

Test for Anthocyanin:

NaOH Test:

A small fraction of extract was treated with 2M NaOH and detected the development of blue green color shows anthocyanin in extract of leaves of *Parthenium hysterophorus*.

Antimicrobial assay

Parthenium hysterophorus leaves extracts were used for the assessment of antimicrobial activity. This experimentally was done by agar well diffusion method. In agar well diffusion method, a pure isolate of each experimental microorganism was culture on agar plates. One plate of microorganism was taken. Under the aseptic conditions, four colonies were transferred into normal saline. 100 µL of the inoculum of each test microorganism was poured onto the agar plates. The agar plates were allowable to dry and wells were prepared of 8 mm. The dried *Parthenium hysterophorus* leaves extracts were reconstituted to the 20% in DMSO. Each extract of 100 µL volume was impelled directly into the wells. The plates were allowed to stand for 1 h at the 40°C for diffusion of the *Parthenium hysterophorus* leaves extract into agar. Now incubation at 37°C for 24 h was done. In this this assay DMSO served as the negative control. Ciprofloxacin was used as positive control for bacteria and amphotericin-B for fungi. The experiments were performed in triplicate. The mean values of the diameter of inhibition zones ± standard deviations were calculated (Aneja *et al*., 2011).

Determination of minimum inhibitory concentration

The minimum inhibitory concentration of various crude extracts for each tested bacterium and fungi was determined by the modified agar well diffusion method (Aneja *et al*., 2011). A twofold serial dilution of each *Parthenium hysterophorus* extract was prepared by first reconstituting the dried extracted *Parthenium hysterophorus* material, 100 mg mLG_1 in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50-0.39 mg mLG_1. A 100 µL volume of each dilution was introduced into triplicate wells of the agar plates already seeded with 100 µL of standardized inoculum of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for inhibition zones. The minimum inhibitory concentration, taken as the lowest concentration of the extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism (Nostro *et al*., 2000; Adeniyi and Ayepola, 2008).The experimental results were repeated thrice in triplicate each time and expressed as Mean ± SD and results were statistically evaluated using Dennett's T-test. p-value less than 0.01 was considered significant.

III. RESULTS

Differential amount of flavonoid contents were present in all the extracts. Comparatively higher amount of flavonoid content was found in *Parthenium hysterophorus* leaf Choloroform, extracts. Positive controls produced significantly sized inhibition zones against the tested bacteria (ranging between5.3 and 31.6 mm) and the yeast (with zone of inhibition ranging between 7.1 and 20.1 mm) and the negative control produced no observable inhibitory effect against any of the test organism. All the tested solvent extracts possessed antibacterial activity against the tested bacterial pathogens except *Pseudomonas aeruginosa* which shows no activity against some extracts. However, all the tested weed extracts did not exhibit much antifungal activity. Ethyl acetate extract has been found very effective showing the maximum zone of inhibition as compared and low MIC against the tested bacterial pathogens.

B. subtilis was found most sensitive pathogen and E. coli was found to be least sensitive pathogen. In the present study, the standard antibiotic, Ciprofloxacin and amphotericin B consistently displayed superior potency when compared with the tested crude extracts. All the obtained results were statistically significant as they showed ($p<0.01$) compared with control.

The antibacterial activity of *Parthenium hysterophorus* extracts on the agar plates varied in ethanol, methanol, acetone, aqueous, petroleum ether, distilled water, chloroform and ethyl acetate extracts. Positive controls produced significantly sized inhibition zones against the tested bacteria and the yeast and the negative control produced no observable inhibitory effect against any of the test organism.

IV. DISCUSSION

Emergence of multi-drug resistance in human pathogenic microorganism as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Man has been using plants either in pure forms or crude extracts, since time immemorial. Bioactive compounds from these plant sources have been isolated and characterized worldwide and systematic screening of plant materials represent an important effort to find some new bioactive compounds with the high therapeutic potential to fight against pathogenic microorganisms. The elucidation of the chemical structures of some of these compounds had led to the synthesis and production of more potent and safer drugs (Parekh and Chanda, 2007; Sharma *et al*., 2011). The potency of weed plants to grow at extreme conditions can be exploited properly for mankind. In the present study, we have investigated the qualitative phytochemical analysis of extract of *Parthenium hysterophorus* L. leaves. Earlier Gupta et al. 1977 reported the presence of Amino acids, Carbohydrates and Saponins in the methanol extract of *P. hysterophorus*. In the present study along with the above mentioned compounds we had found the presence of additional phytochemical constituents. All the tested extracts showed varied amount of antibacterial activity this might be due to the presence of phytochemical constituents. This can partially explain the demonstration of antibacterial activity by the leaves extracts of weeds (Bonjar *et al*., 2004).

In the present study, Ethyl acetate showed the highest activity against the tested organisms, due to better solubility of the active compounds in organic solvents (De Boer *et al*., 2005; Salama and Marraiki, 2010). The effectiveness of the extracts largely depends on the type of solvent used, where the organic extracts provided more powerful antimicrobial activity (Sen and Batra, 2012). Cowan (1999) mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents. Seyydnejad *et al.* (2010) also studied the effect of different alcoholic viz. ethanol and methanol for antimicrobial activity and observed that this difference in the activity between different alcoholic extract is due to the difference between extract compounds in this two extract. Our results are in accordance with other studies (Bhuvaneswari *et al*., 2011; Prakash *et al*., 2012). It has been observed that plant extracts are resistant to *Pseudomonas aeruginosa* due to the permeability barrier afforded by its outer membrane. Also its tendency to colonies in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics.

In present study, the antibacterial activity of plant extract appears to be inhibitory to Gram-positive bacteria. The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria (Kirtikar and Basu, 1968; Sheu and Freese, 1973; Turnbull and Kramer, 1991).

V. CONCLUSION

Several secondary metabolites were analysed in the leaves of weed Parthenium hysterophorus. Since this plant had been used in the treatment of several diseases, the medicinal roles of these plants could be related to such identify secondary metabolites. The current study depicts that the secondary metabolites may contribute in many noteworthy ways for various studies to the pharmaceutical activity. From this study it is concluded that the crude extracts obtained from the leaves of the *Parthenium hysterophorus* showed the antimicrobial activity, indicating that this plant is a good source of antibiotics for their treatment of certain microbial diseases as also experimentally proved by the Sahaj Kaur, Chetan Sharma, Smita Chaudhry and Robina Aman, 2015. As of now, little work has been done on the antimicrobial activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigations are needed such as in vivo studies of *Parthenium hysterophorus* necessary to determine toxicity of the active constituents, their side effects, pharmacokinetics properties to exploit the bioactive principles, for therapeutic utility in treating the bacterial infections. This paper suggests that *Parthenium hysterophorus* and such weed can be exploited to prepare potent antibacterial drugs.

REFERENCES

[1]. A comparative study on antibacterial activity of common weeds. Int. J. Pharm. Biosci., 2: 677-683.
[2]. Adeniyi, B.A. and O.O. Ayepola, 2008. The phytochemical screening and antimicrobial activity of leaf extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* (Myrtaceae). Res. J. Med. Plant, 2: 34-38.
[3]. Ali, E.M.M., A.Z.I. Almagboul, S.M.E. Khogali and U.M.A. Gergeir, 2012. Antimicrobial Activity of *Cannabis sativa* L. Chin. Med., 3: 61-64.
[4]. Aneja, K.R. and C. Sharma, 2010. Antimicrobial potential of fruit extracts of *Elettaria cardamomum* maton (Chhoti: Elaichi) against the pathogens causing ear infection. Pharmacologyonline, 3: 750-756.
[5]. Aneja, K.R., C. Sharma and R. Joshi, 2011. *In vitro* efficacy of amaltas (*Cassia fistula* L.) against the pathogens causing otitis externa. Jundishapur J. Microbiol., 4: 175-183.

[6]. Bhuvaneswari, S., R. Aravind, V. Kaviyarasan, K. Kalaivanan and S.B. Hariram, 2011.
[7]. Bnjar, G.H.S., S. Aghighi and A.K. Nik, 2004. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of South East regions of Iran. J. Biol. Sci., 4: 405-412.
[8]. Christy Gnana Theeba P. and Sasi Kumar R. Journal of Chemical and Pharmaceutical Research, 2015, 7(4):219-225
[9]. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
[10]. De Boer, H.J., A. Kool, A. Broberg, W.R. Mziray, I. Hedberg and J.J. Levenfors, 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol., 96: 461-469.
[11]. Edeoga HO, Okwu DE and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol 2005;47):685-688.URL-http://www.ajol.info/index.php/ajb/article/view/15167)
[12]. Gupta RK, Dutta TR, Patil BD. Chemical investigation of Parthenium hysterophorus. Indian Journal of Pharmacy 1977; 393) : 64-66. URL - http://www.ansfoundation.org/Uploaded%20Pdf/41/137-143.pdf)
[13]. James, L. and J.O. Evans, 1991. Noxious Range Weeds. Westview Press, Boulder, CO., USA., ISBN-13: 9780813383958, Pages: 466.
[14]. Kirtikar, K.R. and B.D. Basu, 1968. Indian Medicinal Plants. Vol. 1, Lalit Mohan Basu, Allahabad, India.
[15]. Kumar, S., A. Mishra and A.K. Pandey, 2013a. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *in vitro* models. BMC Complementary Altern. Med., Vol. 13. 10.1186/1472-6882-13-120
[16]. Kumar, S., A.K. Gupta and A.K. Pandey, 2013b. *Calotropis procera* root extract has the capability to combat free radical mediated damage. ISRN Pharmacol. 10.1155/2013/691372
[17]. Lambert, D.M. and C.J. Fowler, 2005. The endocannabinoid system: Drug targets, lead compounds and potential therapeutic applications. J. Med. Chem., 48: 5059-5087.
[18]. M. Kaur, N.K. Aggarwal and R. Dhiman. 2016. Research Journal of Medicinal Plant 10 (1): 106-112.
[19]. Madureira, A.M., A. Duarte and G. Teixeira, 2012. Antimicrobial activity of selected extracts from *Hakea salicifolia* and *H. sericeae* (Proteaceae) against *Staphylococcus aureus* multiresistant strains. South Afr. J. Bot., 81: 40-43.
[20]. Manikandan, S., S. Ganesapandian, M. Singh, N. Sangeetha and A.K. Kumaraguru, 2011. Antimicrobial activity of seaweeds against multi drug resistant strains. Int. J. Pharmacol., 7: 522-526.
[21]. Meena, A.K., A. Yadav and M.M. Rao, 2011. Ayurvedic uses and pharmacological activities of *Calotropis procera* Linn. Asian J. Traditional Med., 6: 45-53.
[22]. Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli, 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbial., 30: 379-384.
[23]. Parekh, J. and S. Chanda, 2007. *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. Afr. J. Biotechnol., 6: 766-770.
[24]. Prakash, N.K.U., S. Bhuvaneswari, B. Jahnavi, K. Abhinaya and A.G. Rajalin *et al*., 2012. A study on antibacterial activity of common weeds in Northern Districts of Tamil Nadu, India. Res. J. Med. Plant, 6: 341-345.
[25]. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur. Phytochemical screening and Extraction: A Review. IPS 2011; 1 : 98 -106. URL - http://www.ipharmsciencia.com/Dacuments/1/11.pdf)
[26]. R Sasi Kumar; P Balasubramanian; P Govindaraj; T Krishnaveni, Journal of Pharmacognosy and Phytochemistry, 2014, 2 (6), 74 - 78.
[27]. Sachindra, N. and A. Pradhan, 1977. Marijuana Drug Abuse Clinical and Basic Aspects. The C.V. Mosby Co., Saint Louis.
[28]. Sahaj Kaur, Chetan Sharma, Smita Chaudhry and Robina Aman. Antimicrobial Potential of Three Common Weeds of Kurukshetra: An in vitro Study. Research Journal of Microbiology, 2015, 10 (6): 280-287
[29]. Salama, H.M.H. and N. Marraiki, 2010. Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. Saudi J. Biol. Sci., 17: 57-63.
[30]. Sanguri, S., S. Kapil, P. Gopinathan, F.K. Pandey and T. Bhatnagar, 2012. Comparative screening of antibacterial and antifungal activities of some weeds and medicinal plants leaf extracts: An *in-vitro* study. Elixir Applied Bot., 47: 8903-8905.
[31]. Sen, A. and A. Batra, 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. Int. J. Curr. Pharmaceut. Res., 4: 67-73.
[32]. Seyydnejad, S.M., M. Niknejad, I. Darabpoor and H. Motamedi, 2010. Antibacterial activity of hydroalcoholic extract of callistemon citrinus and albizia lebbeck. Am. J. Applied Sci., 7: 13-16.

[33]. Sharma, C., K.R. Aneja and R. Kasera, 2011. Screening of *Berberis aristata* DC. for antimicrobial potential against the pathogens causing ear infection. Int. J. Pharmacol., 7: 536-541.
[34]. Sharma, R., G.S. Thakur, B.S. Sanodiya, A. Savita, M. Pandey, A. Sharma and P.S. Bisen, 2012.
[35]. Sheu, C.W. and E. Freese, 1973. Lipopolysaccharide layer protection of gram-negative bacteria against inhibition by long-chain fatty acids. J. Applied Bacteriol., 115: 869-875.
[36]. Therapeutic potential of *Calotropis procera*: A giant milkweed. Int. J. Pharm. Biol. Sci., 4: 42-57.
[37]. Trease GE, Evans WC. Text book of Pharmocognosy. 13th eds). Alden Press; Oxford 1989; 512-513.
[38]. Turnbull, P.C.B. and J.M. Kramer, 1991. Manuals of Clinical Microbiology. In: Bacillus, Barlows, A., W.J. Hausler, H.D. Herrmann, H. Isenberg and H.J. Shadomy (Eds.). 5th Edn., American Society for Microbiology, Washington DC.
[39]. Yadav RNS and Munin Agarwala. Phytochemical analysis of some medicinal plants. Journal of Phytology 2011; 312) : 1014. URL http://journalphytology.com/index.php/phyto/article/viewfile/9530/5167)

IOSR Journal of Engineering (IOSRJEN) is UGC approved Journal with Sl. No. 3240, Journal no. 48995.

Saksham Khusharia "Photochemical Screening with Estimation of Total Flavonoid Content in Parthenium Hysterophorus and In Vitro Analysis of Antimicrobial Activity." IOSR Journal of Engineering (IOSRJEN), vol. 08, no. 7, 2018, pp. 01-07.