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Microbial Profile of the Phyllosphere and the Antimicrobial potency of *Ficus vogelii* extracts

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Abstract

Ficus vogelii is a member of fig genus that belongs to the family of moraceae. The plant is available in many parts of Africa, and it has been commonly used to treat high blood pressure, diarrohea and diabetes. This study was aimed at providing additional knowledge to the biological roles and importance of the plant by evaluating its antimicrobial activities and prevalence of microorganisms on the phyllosphere of the plant. The plant's leaves were prepared and extracted with both ethanol and n-Hexane. The plant's extracts were screened against *Escherichia coli Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis* and results showed the methanolic extract could not inhibit the selected microorganisns while the n-Hexane extract was able to inhibit the microorganisms at higher concentrations with *Bacillus subtilis* being the most susceptible of the bacteria. The antimicrobial activities of the plant were extractant and concentration dependent. The isolated microorganisms (bacteria) were identified by Gram staining technique and biochemical profiling. Both Gram positive and Gram negative bacteria were isolated from the phyllosphere of the *Ficus vogelii* (leaf). Majority of the isolates were Gram positive cocci (87%) while minority was Gram negative (13%). Biochemical tests revealed the presence of *Veillonella sp* (6%), *Rhodococcus sp* (6%), *Acinetobacter parvus* (6%), *Micrococcus sp* (13%), *Heloccocus pyogens* (6%), *Sarcina ventriculi* (6%), *Peptostroptococcus sp* (13%) and *Staphylococcus aureus* (44%). *Staphylococcus aureus* (44%) was the most predominant isolated bacteria on the plant's leave. This research therefore provides background information on the microbial profile of the leaf and antimicrobial activities of *Ficus vogelii* extracts.

Keywords: Antimicrobial activities; *Ficus vogelii*; phyllosphere; Micrororganisms; Isolation; ethnomedicine

1. INTRODUCTION

Pathogenic microorganisms have been known to account for high mortality rates most especially in tropical regions of the world. The existence of pathogenic microorganisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis Vibrio cholera, Shigella dysenteriae, Salmonella typhi* etc. in both aquatic and terrestrial environments poses threats to human health as they are capable of causing diverse infectious diseases (Preeti et al. 2010; Kuete et al., 2011; Sarg et al. 2011). Despite recent advances in the field of microbiology, incidence of epidemics due to drug resistant micro-organisms and emergence of unknown pathogenic microbes have raised enormous public health concerns (Pinner et al., 1996). Most of the commercially available drugs for combating microorganisms are synthetic and expensive. These issues have led to recent exploration of natural products like plants as effective, efficient and affordable alternative to combating pathogenic microorganisms.

Plants are rich phytochemicals which are of a great biological importance (Egbuna et al., 2011; Johnson et al., 2012). The major importance of plants lies in their ethnomedicinal applications for treating and managing human diseases and ailments (Singh, 2015). Traditional medicine derived from plant sources have been identified as the most affordable and easily accessible source of treatment in the primary health care system most especially in rural and poor communities. The potential use of plants for solving human oxidative stress related diseases and disorders such as diabetics, cancer and skin hyperpigmentation have been documented by several researchers (Hu et al., 2002; Shetti et al., 2012; Ahmed et al., 2013; Popoola et al., 2015; Wesam Kooti et al., 2016). The efficacy of plants to combat diseases such as dysentery, typhoid, cholera, hepatitis etc., caused by pathogenic microorganism has also been reported (Ghani, 1998; Hossen, 2003). Common plants which have been reported to possess antimicrobial activities include *Triclisia subcordata (Oliv), Psydrax subcordata,* pumpkin (cucurbit), *Heinsia crinita* (Ajibesin et al., 2003; Abo et al., 2011; Anokwah et al., 2016; Asif et al., 2017). Several *Ficus* species such as *Ficus religiosa, Ficus lyrata, Ficus polita, Ficus racemosa* and *Ficus deltoidea* among others have been evaluated and found to possess antimicrobial activities Uma et al., 2009; Rizvi et al., 2010; kuete et al., 2011; Murti and Kumar 2011; Suryati et al., 2011;.

Ficus Vogelii belong to the family of Moraceae and it is a member of the fig genus found mostly in the Guinea savannah vegetation belt of West and Central Africa. In the middle belt of Nigeria, it serves as vegetable for preparing different types of dishes (Bamikole et al., 2004). *Ficus Vogelii is* known for its ethnomedicinal application most especially in the treatment of diabetes, anorexia and anemia (Igile et al. 2015). Scientific investigation of the plant for its acclaimed therapeutic purposes include its antidiabetic Igile et al. (2015), anti-ulcer Ezemagu et al., (2019), anti-anaemia Bamikole et al., (2004) and Antihepatotoxic potentials Egbuna et al., (2011). To our best of knowledge, the antimicrobial activities of *Ficus Vogelii* have not been established. This research work is therefore aimed at investigating the antimicrobial activities of *Ficus Vogelii* extracts against some selected pathogenic microorganisms and establishing a fact for possible use of the plant to inhibit pathogenic microorganisms. In addition to investigating the antimicrobial potentials of the leaf extracts, the phyllosphere of the plant's leaf was evaluated for presence of microorganisms. This study further provides insight on microbial life on phyllosphere of *Ficus Vogelii*.

Figure 1: Leaf of *Ficus Vogelii* in a sterile bag

2. Materials And Methods

2.1. Sample Collection

2.1.1. Collection of *Ficus vogelii*

The leaves of a healthy-looking *Ficus vogelii* growing in an area that is physically distant from foot traffic (to avoid contamination) was collected at the Faculty of Agriculture, Ekiti State University Ado-Ekiti, Ekiti State, Nigeria and stored in a sterilize bag prior to further analysis. The plant was identified by the Herbarium curator at the Department of Plant Science and Biotechnology, Ekiti State University.

2.1.2. Collection of test organisms

Test organisms (bacteria) used for antimicrobial susceptibility test were reference isolates of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 29213) and *Bacillus subtilis* (NCTC 8253) obtained from the culture bank of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria.

2.2. Isolation and Identification of Microorganisms

The leaf was sonicated in an ultrasonic bath for 5 minutes and serial dilutions were made in duplicates. 0.1ml of the each dilution was plated out on Tryptone soy agar containing 100ug/ml of cycloheximide. Initial isolation from the dilutions was done using spread plate method following the subculture of isolates from various dilutions. Gram staining and biochemical tests were conducted for all the isolates in order to determine the probable organisms using established standard procedures described by Holt et al. (1994) in Bergey's manual of determinative bacteriology

2.3. Leaves preparation

The leave samples were washed and air-dried at room temperature for seven (7) days. The dried plant leaves sample was crushed, pulverized and homogenized into fine powder using an electric blender. Then the sample was weighed and stored in a sterile bottle prior to analysis.

2.4. Extraction

The pulverized plant material was extracted with n-Hexane (nH) and methanol (Me). 50 g of the powdered plant material was soaked in 1000 ml each of nH and Me for 48 hours. The extracts were filtered and concentrated at 50 ^{o}C with the aid of rotary evaporator. Both extracts were stored in different sterile sample bottles prior analysis.

2.5 *In vitro* antimicrobial activities

The *in vitro* antimicrobial activities of the leaf extracts were carried out by well diffusion method using Nutrient agar Cooper *et al.* (2002) on the selected microorganisms (*S. aureus, E. coli, B. Subtilis and P. aeruginosa).* Different concentrations (12.5, 25 and 50 mg/ml) of each extract were prepared by diluting in sterilized water; a net concentration (100 %v/v) of extract was also used. Nutrient agar plates were prepared for culturing of the organisms and each plate was properly inoculated with each test organism using streaking method (Okeke et al., 2018). Wells with diameter of 20mm were bored using a sterile cork-borer and each well was filled with 1ml different concentrations of the each extract (to prevent overlapping of the inhibition zones, a distance was maintained from the edges of the plates) and the plates were incubated for 24 hrs at 37 °C. The inhibition zones were measured and results recorded. Streptomycin (ST) was used as the control.

3. Results And Discussion

3.1. RESULTS

Figure 2: colonies of isolated microorganisms

3.1.1. Microbial Staining and Biochemical Profiles of *Ficus vogelii* Phyllosphere

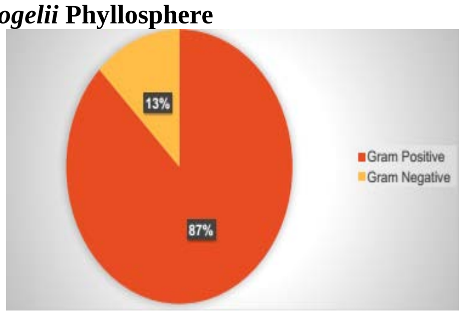

Figure 3: Microbial staining profile of *Ficus vogelii* Phyllosphere

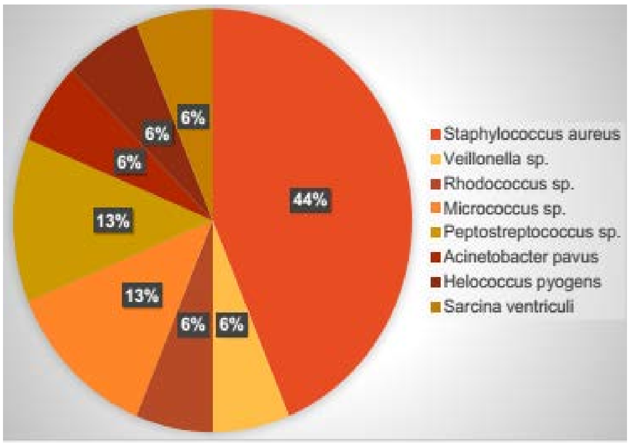

Figure. 4: Microbial biochemical profile of *Ficus vogelii* Phyllosphere

3.1.2. Antimicrobial Activities of the *Ficus vogelii* Extracts

Figure 5: Antimicrobial activities of the methanolic extract and control

Table 1: Antimicrobial activities of the *Ficus vogelii* methanolic extract (FMeE) and control

Sl.No	Concentration (mg/ml)	Zone of inhibition (mm)			
		S.A	B.S	P.A	E.C
1	100	0.00	0.00	0.00	0.00
2	50	0.00	0.00	0.00	0.00
3	25	0.00	0.00	0.00	0.00
4	12.5	0.00	0.00	0.00	0.00
Control	10	22.0	22.0	18.0	21.0

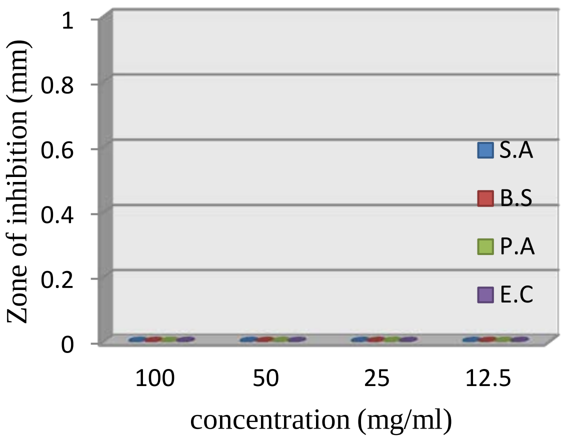

Figure 6: Antimicrobial activities of the methanolic extract of the *Ficus vogelii* (FMeE)

Table 2: Antimicrobial activities of the *Ficus vogelii* n-Hexane extract (FnHE) and control

Sl.No	Concentration (mg/ml)	Zone of inhibition (mm)			
		S.A	B.S	P.A	E.C
1	100	10.00	8.00	12.00	4.40
2	50	8.00	4.20	7.00	0.00
3	25	4.00	0.00	4.00	0.00
4	12.5	0.00	0.00	0.00	0.00
Control	10	22.00	22.00	18.00	21.00

Key: S.A: *Staphylococcus aureus; P.A: Pseudomonas aeruginosa;* E.C: *Escherichia coli*; B.S: *Bacillus subtilis;* Control: Streptomycin; FMeE: *Ficus vogelii* methanolic extract; FnHE: *Ficus vogelii* n-Hexane extract.

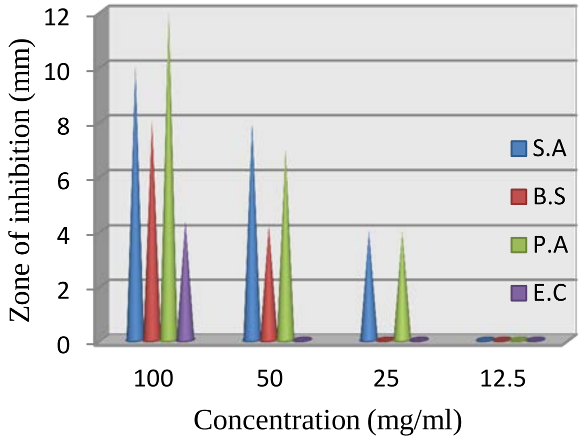

Figure 7: Antimicrobial activities of the n-Hexane extract of the *Ficus vogelii* (FnHE)

3.2. DISCUSSION

3.2.1. Microbial Load of Fresh Leave of *Ficus vogelii* (Gram Staining and Biochemical Profiles)

The phyllosphere is an interface of plant above-ground level which acts as natural habitat for microorganisms (Lindow and Leveau, 2002). Most plants host diverse communities of microorganisms which are either beneficial to the plant or pathogenic. Microorganisms present in plant's phyllosphere usually include: protists, bacteria, fungi and archea. In this research, the leaf of the *Ficus vogelii* was evaluated for the prevalence of microorganisms. The isolated microorganisms (bacteria) were identified by Gram staining technique and biochemical profiling. Both Gram positive and Gram negative bacteria were isolated from the phyllosphere of the *Ficus vogelii* leaf. Majority of the isolates were Gram positive cocci (87%) while minority was Gram negative (13%) (Figure 3). Biochemical tests revealed the presence of *Veillonella sp* (6%), *Rhodococcus sp* (6%), *Acinetobacter parvus* (6%), *Micrococcus sp* (13%), *Heloccocus pyogens* (6%), *Sarcina ventriculi* (6%), *Peptostreptococcus sp* (13%) and *Staphylococcus aureus* (44%) (Figure 4). *Staphylococcus aureus* (44%) was the most predominant isolated bacteria on the plant's leave. The predominance prevalence of *Staphylococcus aureus* (44%) in the phyllosphere of the plant shows that they are natural inhabitants of the phyllosphere of *Ficus vogelii* and this is confirmed by the previous research work reported by Babalola (2013) in which *Staphylococcus aureus* was the most prevalent isolate (60%) from both the phyllosphere of *Ficus sycomorus*.

3.2.2. Antimicrobial Activities of the *Ficus vogelii* Extracts

The *in vitro* antimicrobial activities of the MeE against the selected bacteria (*Escherichia coli Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis*) showed that the bacteria were resistant to the extract (Figure 6). The zone of inhibition (0) measured for all the isolates showed no activity of the extract (Table 2). The inability of the extract to inhibit the microorganisms could be as a result of the inability of the Methanol to extract the bioactive compound which could be potent against the microorganisms. It is well known that plants contain active bioactive compounds (phytochemicals) that could inhibit microorganisms (Murti and Kumar 2011; Asif et al., 2017). The antimicrobial activities of plants' phytochemicals follow series of inhibition mechanisms. Some of these phytochemicals may possess lipohilic properties, proton efflux inhibition activities and charged protons as their way of inhibiting microorganism (Gutneckt and Walter 1981; Salmond *et al*., 1984; Sanchez et al., 2010). Microorganisms also have different mechanisms of defense to active plant phytochemicals and activities of these bioactive compounds in plants are bio-selective and concentration dependent. This implies that microorganisms can only be inhibited upon exposure to certain sensitive compounds which could overcome their resistance and mechanisms of defense when the appropriate concentration of the compound is used. Thus, the inability of the FMeE to inhibit any of the microorganisms even at the tested concentrations (12.5, 25, 50 and 100 mg/mg) showed that the extract doesn't contain the required active phytochemicals to overcome the resistance and defense mechanisms of the microorganisms. Similar research to this work showing that microorganisms were resistant to plant extracts has been reported by Oyeleke et al. (2008); Adesina et al. (2010). Streptomycin (control) was active against the selected bacteria.

Contrarily, the results of the antibacterial activities of the FnHE showed that the extract was potent against the selected microorganisms (Table 3) and the activities increased with increase concentration of the extract. The extract showed no activity against all the test organisms at the lowest concentration (12.5 mg/ml). The FnHE only showed activity at the concentration of: (100 mg/ml) against *E. coli*; 50 and 100 mg/ml against *P. aeruginosa* and *B. subtilis*; 25, 50 and 100 mg/ml against *S. aureus* (Figure 7). These results showed that the antibacterial activities of the FnHE were concentration dependent. Also, the extract was active against all the selected microorganisms at 100 mg/ml. The activities of the FnHE may be due to the ability of the n-Hexane to extract active phytochemical(s) which could overcome the defense mechanisms of the bacteria. Increase of the antimicrobial activities of the extract with increased concentration is an indication that more of the active phytochemicals are needed to overcome resistance. This implies that the bioactive compounds may possess lipohilic properties which allow their permeation into the lipoid layer of the cell membrane and exerting cytotoxicity by their accumulation in the cells of the microorganism. The accumulation of the bioactive com-pounds could inhibit the active effluxes or reduces their activities in the cells of the micro-organisms. Thus, higher concentration of the bioactive compounds would imply higher antimicrobial activities. The results from this work corroborate to previously reported works by Abo et al. (2011); Koona and Rao (2012); Anokwah et al. (2016).

4. CONCLUSION

This study has explored the prevalence of microorganisms in the phyllosphere of *Ficus vogelii* (leaf) as well as the antimicrobial activity of the plant's leaf extracts. The results of this study showed that several bacteria (both Gram positive and gram negative) inhabit the phyllosphere of *Ficus vogelii.* The most prevalence of these is the Gram positive bacteria. Also, the antimicrobial activities of the methanolic extract of the plant's leaf showed no activities while that of the n-Hexane showed good activities at high concentration as the extract may contain the necessary active bioactive compounds for this purpose. This research provides background information on the antimicrobial activities of *Ficus vogelii* extracts. Also, this research forms a baseline work for further research on antimicrobial activities of *Ficus vogelii* and provides additional information on the existing data on biological roles of the plant. Thus, we recommend that further research be carried on evaluation of the antimicrobial activities of various parts of *Ficus vogelii* to ascertain the use of the plant as an effective antimicrobial agent. It would also be prudent to determine the antimicrobial susceptibility profiles of the isolated microorganisms from the phyllosphere of the plant against different solvent extracts of the leaves or other parts of the plant in other to determine their resistance profile.

Conflict of Interest

The authors declare that there are no conflicts of interest

REFERENCES

1. Rizvi, W., Rizvi, M., Kumar, R., Kumar, A., Shukla, I and Parveen M. (2010). Antibacterial activity of *Ficus lyrata* -An *in vitro* study. Int. J. Pharmacol. 8(2):7.
2. Preeti, R., Devanathan, V, and Loganathan, M. (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food Borne pathogens. Advances Biol. Res. 4:122–125.
3. Sarg, T.M., Abbas, F.A., El-Sayed, Z.I. and Mustafa, A.M. (2011). Two new polyphenolic compounds from *Ficus retusa* L."variegata" and the biological activity of the different plant extracts. J. Pharmacog. Phytother. 3:89–100.
4. Wesam, K, Maryam, F., Zahra A., Damoon, A and Majid, A. (2016), The role of medicinal plants in the treatment of diabetes: a systematic review *journal of Electronic Physician* Volume: 8, Issue: 1, Pages: 1832-1842, DOI: http://dx.doi.org/10.19082/1832.
5. Hu, H., Ahn, N.S., Yang, X., Lee, Y.S and Kang, K.S. (2002) *Ganoderma lucidum* extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int J Cancer 102: 250-253.
6. Uma, B., Prabaker, K and Rajendran, S. (2009). In vitro antimicrobial activity and phytochemical analysis of *Ficus religiosa* and *Ficus benghalensis* L., against enterotoxigenic *E. coli*. Food Chemical toxicology (11)2842–6.

7. Murti, K and Kumar, U. (2011). Antimicrobial activity of *Ficus benghalensis* and *Ficus racemosa* roots L. Am. J. Microbiol. 2:21–24.
8. Suryati, H Nurdin, Dachriyanus, and Lajis M.N.H. (2011). Structure elucidation of antibacterial compound from *Ficus deltoidea* Lack leaves. Indo. J. Chem. 11:67–70.
9. Ajibesin, K.K., Ekpo, J.B., and Bala DN (2003). "Comparative pharmacognostic and antimicrobial studies on leaves of *Heinsia crinita"*, Global J. Med. Sci. 2: 49-57.
10. Sanchez, E., Garcia S., Heredia N. (2010). Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae*. *Applied Environmental Microbiology*. 76 6888–6894.
11. Koona, S.J and Rao, B.S. (2012). *In vitro* evaluation of antibacterial activity of crude extracts of *Ficus benghalensis* Linn., the banyan tree leaves. Indian J. Natural Products Resour. 3:281–284.
12. Lindow, Steven E; Leveau, Johan (2002) "Phylosphere microbiology". Current Opinion in Biotechnology 13 (3): 238-243.
13. Salmond, C.V., Kroll, R.G. and Booth I.R. (1984). The effects of Food Preservatives on pH Homeostasis in *Escherichia coli*. *Journal of Central Microbiology* 130 (11): 2845-50.
14. Gutknecht, J., and Walter, A. (1981). Histamine, theophylline and tryptamine transport through lipid bilayer membranes. Biochem. Biophys. Acta. 649: 149-154 [Pubmed]
15. Kuete, V, Kamga J, Sandjo LP, Ngameni B, Poumale HMP, Ambassa P, Ngadjui BT (2011). Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl. (Moraceae). BMC Complementary and Alternative Medicine 11:6.
16. Pinner RS, Teutsch L,Simonsen L, Klug J, Graber M, Berkelman R (1996). "Trends in infectious diseases mortality in the United States", J. Am. Med. Ass., 275: 189-193.
17. Singh R. (2015). Medicinal Plants: A Review. *Journal of Plant Sciences*. Special Issue: Medicinal Plants. Vol. 3, No. 1-1, pp. 50-55.
18. Shetti A, Sanakal R and Kaliwal B. (2012). Antidiabetic effect of ethanolic leaf extract of Phyllanthus amarus in alloxan induced diabetic mice. Asian J Plant Sci Res; 2(1): 11-5.
19. Ahmed, M., Khan, M.I., Khan, M.R., Muhammad, N., Khan, A.U and Khan, A.R. (2013) Role of Medicinal Plants in Oxidative Stress and Cancer. *OMICS Publishing Group* 2:641 doi:10.4172/scientificreports.641
20. Popoola, O.K, Marnewick J.L, Rautenbach F, Ameer F, Iwuoha EI, et al. (2015) Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. Molecules 20(4): 7143-7155.
21. Asif, M., Naqvi, S.A.R., Sherazi, T.A., Ahmad, M., Zahoor, A.F., Shahzad, S.A., Hussain, Z., Mahmood, H and Mahmood, N., (2017). Antioxidant, antibacterial and antiproliferative activities of pumpkin (cucurbit) peel and puree extracts-an *in vitro* study. Pak. J. Pharm. Sci. 30(4), 1327-1334.
22. Anokwah, D., Mensah, A.Y., Amponsah, I.K., Mireku, E.A., and Mintah. (2016). Anti-inflammatory, antioxidant and antimicrobial activities of the stem bark of *Psydrax subcordata.* Der Pharmacia Lettre, 8 (20):21-28
23. Bamikole, M.A, Ikhatua, U.J, Arigbede, O.M, Babayemi, O.J, Etela .I. (2004). An evaluation of the acceptability as forage of some nutritive and anti-nutritive components and of the dry matter degradation profiles of five species of *Ficus*. Trop Anim Health Prod; 36:157-167.
24. Igile, G. O., I.C. Utin, I.A., Iwara, B.I.A. Mgbeje and P.E (2015). Ebong Ethanolic Extract of Ficus vogelii Ameliorates Dyslipidemia in Diabetic Albino Wistar Rats *Int. J. Curr. Res. Biosci. Plant Biol*., 2(8): 87-96
25. Ezemagu, U.K., Akunna, G.G., Egwu, O.A, Uzomba, G.C & Nwite, K.N (2019). Comparing *Ficus Vogelii* leaf extract and omeprazole as therapy and prophylaxis for aspirin-induced gastric ulcer in wistar rat. *Biomedical Research; 30 (5): 697-703*
26. Cooper, R., Molan, P and Harding, K (2002). "The sensitivity to honey of gram positive cocci of clinical significance isolated from wounds". Journal of Applied microbiology, 93(5): 857 – 863.
27. Okeke, O., Okeke, M. U., Ezejiofor, C.C. and Ndubuisi, J.O. (2018). "Antimicrobial Activity of Honeys from Nsukka andN Ugwuaji in Enugu State, on Selected Pathogenic Bacteria Isolated from Wound" Advances in Analytical Chemistry, 8(1): 6-9.
28. Babalola Michael Oluyemi (2013). Multidrug Resistant *Staphylococcus* Isolates from the Phyllosphere and Rhizosphere of *Ficus sycomorus* LinnWorld Rural Observations 5(3)
29. Oyeleke, S. B., Dauda, B. E. N. & Boye, O. A (2008). Antibacterial activity of *Ficus capensis* African Journal of Biotechnology Vol. 7 (10), pp. 1414-1417
30. Adeshina, G.O, Okeke, C.E, Osuagwu, N.O & Ehinmidu, J.O. (2010). Preliminary in-vitro antibacterial activities of ethanolic extracts of Ficus sycomorus Linn. And Ficus platyphylla Del. (Moraceae). African Journal of Microbiology Research; 4(8):598-601.
31. Ghani, A, (1998). Medicinal plants of Bangladesh: chemical constituents and uses. Asiatic society of Bangladesh, Dhaka, 460p.
32. Egbuna, P.A.C., Joshua, P.E., Chigbo, M.U., (2011). Antihepatotoxic effect of *Ficus Vogelii* ethanol leaf extract on the liver function indices of $CC1_4$-induced hepatotoxicity in rats. J. Amer. Sci. 7(6), 158-163.
33. Egbuna, P.A.C., Joshua, P.E., Chigbo, M.U., (2011).Antihepatotoxic effect of *Ficus Vogelii* ethanol leaf extract on the liver function indices of $CC1_4$-induced hepatotoxicity in rats. J. Amer. Sci. 7(6), 158-163.
34. Hossen, A.B.M, (2003), medicinal plants, fruits, flowers, tree cultivation and uses. 2/F, 2/1 Mirpur Dhaka.192p
35. Johnson U.J. T., Ochigbo V., Udiba U. U., Gauje, B., (2012). Effect of activity directed administration of ethanolic extracts of *Nauclea latifolia* on the body and organ weights of hypertensive induced albino wistar rats. Int. J. Sci. Technol. 2(6), 382-385.
36. Abo, K. A., Lawal, I. O., Ogunkanmi, A. (2011). Evaluation of extracts of *Triclisia suboardata* Oliv and *Heinsia crinita* (Afz) G. Taylor for antimicrobial activity against some clinical bacterial isolates and fungi. *African journal of pharmacy and pharmacology*, 5(2). pp. 125-131.
37. Holt, J.G., Kreig, N.R., Sneath, P.H.A and Staley, J.T (1994). Bergey's Manual of Determinative Bacteriology, The William and Wilkins Co., Baltimore, Md, USA, 9^{th} edition.