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ANTIMICROBIAL AND ANTIOXIDANT SCREENING OF METHANOLIC EXTRACT FROM LEAF AND FLOWER PARTS OF *WOODFORDIA FRUTICOSA*.

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The main objective of this study was to assess antimicrobial and antioxidant activity of flower and leaf samples of *Woodfordia fruticosa*. Antimicrobial assay of the crude extracts was performed against six tested pathogenic strains including two Gram-positive bacteria viz. *Staphylococcus aureus* and *Micromonospora* sp., two Gram negative bacteria viz. *Staphylococcus epidermis* and *Zymomonas mobilis* and two fungi, *Alternaria solani* and *Fusarium culmorum* by Agar-well diffusion method. The MIC values were determined by broth dilution method. The antioxidant activity of the extracts of the flowers and leaves of *Woodfordia fructicosa* were investigated by using DPPH scavenging assay and reducing power of the extract. Flowers extracts of the plant has got profound and significant percent (%) DPPH scavenging activity (93.48 ± 0.26) at 200 µg/ml concentration followed by leaves (84.17 ± 0.06) at 200 µg/ml concentration. Antimicrobial screening revealed that the flower parts of the plant showed better antimicrobial activity against micro - organisms as compared to leaf part. Maximum Zone of inhibition was observed in the flower extracts against *Staphylococcus aureus* (22.4 ± 0.86 mm) amongst the bacteria species and against *Alternaria solani* (23.5 ± 0.15 mm) amongst the fungal species.

Keywords : Antimicrobial; Antioxidant, DPPH; *Woodfordia fruticosa*,

Introduction

Bioactive substances lead to the discovery of new compounds that could be used to formulate new and most potent antimicrobial drugs to overcome the problem of resistance to the currently available medicines. Now a day's, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In India, addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity,

immune suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances.

Plants have been used long before the discovery of antibiotics as remedies for a number of human diseases. They contain a great array of secondary metabolites having therapeutic value. Traditional healers often referred as herbal healers, from various parts of the world use plants as anti-infective agents. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [1-3]. Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to plant tissue or extracts which are in contact with them.

Plants contain a wide variety of antioxidant molecules, such as flavonoids, anthocyanins, carotenoids, which scavenges a variety of free radicals. Although the body has effective defence systems that protect it against oxidative stress, the capacity of these protective systems decreases with aging creating a need to provide the body with a constant supply of phytochemicals through dietary supplements [4]. The majority of the antioxidant activity of plants may be from phenolic compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin, and epicatechin. Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Free radicals and related species have attracted a great deal of attention in recent years. Damage induced by ROS (Reactive oxygen species) includes DNA mutation, protein oxidation and lipid peroxidation, contributing to the development of cancer, diabetes; atherosclerosis, inflammation and premature aging [5]. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species.

Woodfordia fruticosa commonly called as Dhavari, Dhatki, Dhawai is spreading evergreen shrub belongs to the family Lythraceae. It is native of Asia and Africa and it is widely distributed in throughout the world [6]. Various natural compounds like tannins, phenols, flavonoids, terpenoids/steroids, carbohydrates, etc. have been present in this plant. By tradition, its entire part is valuable, but it is commonly cultivated for its leaves and flowers. It is very useful in the case of fever, dysentery, headache, diarrhea, haemorrhoids, liver diseases, piles, disorders of mucous membranes, leucorrhoea, menorrhagia, skin diseases, burning sensations, herpes, ulcer and wounds by playing acting role of stimulant, astringent and tonic [7,8]. Flowers of this plant are the most effective fermentation agents in ayurvedic medicines [9]. It is used both internally as well as externally. The leaves of *Woodfordia fruticosa* possess antibiotic activity *in vitro* against *Micrococcus pyogenes* var. *aureus*, sedative properties [10], anti-tumour activity [11], antipyretic [8] and anti-inflammatory activity as well. So, the aim of the present study was to determine and compare the antimicrobial and antioxidant activity of leaf and flower extracts of *Woodfordia fruticosa*, a medicinally valuable indigenous plant.

Materials and Methods

(i) Plant material: The samples for antimicrobial screening were collected from Haridwar, Rishikesh and Jhadol (Udaipur) and voucher specimens deposited in the Rajasthan University's Herbarium (RUBL20635). They were established in the

nursery of Department of Botany, University of Rajasthan and used in the study.

(ii) Preparation of extract: Flower and leaf samples of the plant were dried in the dark at room temperature, powdered and extracted by soxhlet extraction method using methanol as solvent. Afterwards, the solvent was distilled under reduced pressure in a rotary vacuum evaporator until the extracts became dry. The crude evaporated plant extracts were dried at room temperature for 5-30 days. Then 50 mg of each crude plant extract was dissolved in 1 mL (1,000 microlitres) of the solvent to give a final concentration of crude extract in solvent of 50 mg/mL. Then, this extract is used for both antimicrobial and antioxidant assay.

(iii) Test microorganisms: Six different strains of microorganisms were used in the screening process including two Gram-positive bacteria viz. *Staphylococcus aureus* (MTCC 3160) and *Micromonospora* sp. (MTCC 3286), two Gram negative bacteria viz. *Staphylococcus epidermis* (MTCC 3615) and *Zymomonas mobilis* (MTCC 88) and two fungi, *Alternaria solani* (MTCC 2101) and *Fusarium culmorum* (MTCC 349), collected from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacteria were grown in nutrient broth (Himedia, M002) at 37^{o}C and maintained on nutrient agar slants at 4^{o}C and fungal cultures were grown and maintained on potato dextrose agar slants at 4^{o}C.

(iv) Antimicrobial activity: Antimicrobial assay of the crude extracts was performed against six tested pathogenic strains by Agar-well diffusion method. The bacterial strains were grown on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). The suspension was used to inoculate 90 mm diameter petriplates. Wells (6 mm diameter) were punched in the agar and filled with the test samples (crude methanol extracts of flower and leaf samples of plant) to get different concentrations viz. 15, 25, 35, 45, 55 µl of the extract. Ampicillin was used as a standard for anti bacterial assay and Flucanazol for antifungal assay. Plates were incubated at 37±2^{o}C for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters and the activity index was calculated for each of these. The experiments were conducted in triplicate. The same method was followed for testing antifungal activity using potato dextrose agar medium.

$$\text{Activity index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

(v) Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method [12]. For broth dilution, 1ml of standardized suspension of a strain (106 cfu/ ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37^{o}C for 24h (for bacterial strains) and 25^{o}C for 48hours (for fungal strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of microorganisms is known as MIC.

(vi) Antioxidant activity: Antioxidant activity (DPPH free radical scavenging

Table 1. Antimicrobial screening (Zone of Inhibition) of methanolic extract from leaf and flower parts of *Woodfordia fruticosa*

Gram -negative Bacteria		**Flower**	**Leaf**	**Standard**
Zymomonas .mobilis *MTCC 88*	IZ	19.2±0.43	13.4±0.31	22.3
	AI	0.87	0.61	
Staphylococcus .epidermis MTCC 3615	IZ	15.7±0.30	17.2±0.44	19.6
	AI	0.8	0.87	
Gram-positive Bacteria				
Micromonospora sp. MTCC 3286	IZ	20.3 ±0.89	10.1 ±0.13	19.9
	AI	1.02	0.51	
Staphylococcus. aureus MTCC 3160	IZ	22.4 ±0.86	18.9± 0.11	22.1
	AI	1	0.85	
Fungi				
Fusarium culmorum MTCC 349	IZ	Nil	14.1±0.12	17.2
	AI	Nil	0.82	
Alternaria solani MTCC 2101	IZ	23.5 ±0.15	Nil	20

IZ= Inhibition zone (in mm) includes the diameter of disc (6 mm);
Standards: Antibacterial- Ampicillin (1.0 mg/ml), Antifungal - Flucanazol (1.0 mg/ml);
AI- Activity Index = IZ of test sample / IZ of standard.
Values are mean of triplicate readings (mean ± S.D).

activity) of methanolic extracts of *Woodfordia fructicosa* flowers and leaves and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 50 - 200 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. Methanol served as the blank and an equal amount of methanol and DPPH (without extracts) served as control. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = A-B/A x 100
Where

A = optical density of the control (not treated with extract) and

B = optical density of the sample

Table 2. Antimicrobial activity (MIC) of *Woodfordia fruticosa* Linn. (Kurz.)

Gram -negative Bacteria	**Flower MIC (µg/ml)**	**Leaf MIC(µg/ml)**	**Standard MIC (µg/ml)**
Zymomonas mobilis MTCC 88	13.2	9.27	15.2
Staphylococcus epidermis MTCC 3615	18.8	20.44	23.5
Gram-positive Bacteria			
Micromonospora sp. MTCC 3286	33.86	16.9	33.2
Staphylococcus aureus MTCC 3160	20.6	17.51	20.6
Fungi			
Alternaria solani MTCC 2101	19.38	nil	19

Table 3. Percent (%) DPPH scavenging activity of the flower and leaf extracts of *Woodfordia fructicosa*

Extracts	**Concentration (µg/ml)**	**DPPH Assay MEAN (SEM)**
Flower	50	78.14 ± 0.30
	100	82.32 ± 1.682
	200	**93.48 ± 0.26**
Leaves	50	74.03 ± 0.79
	100	80.20 ± 0.15
	200	84.17 ± 0.06
Standard (Ascorbic acid)	50	70.16 ± 0.83
	100	72.29 ± 1.53
	200	69.28 ± 0.31

Results and Discussion

Antimicrobial activity: The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to medicine. Plant based antimicrobials have enormous therapeutic potential as they can survive the purpose without any side effects that are often associated with synthetic antimicrobials; continued further research and exploration of plant derived the development of a phytomedicine to act against microbes. Plant based antimicrobial represents the vast untapped source for antimicrobials is needed today. Medicinal plants are an important source for the development of potential, new chemotherapeutic drugs and the in *vitro* antibacterial test form the basis [13]. Many of the studies were useful in identifying the

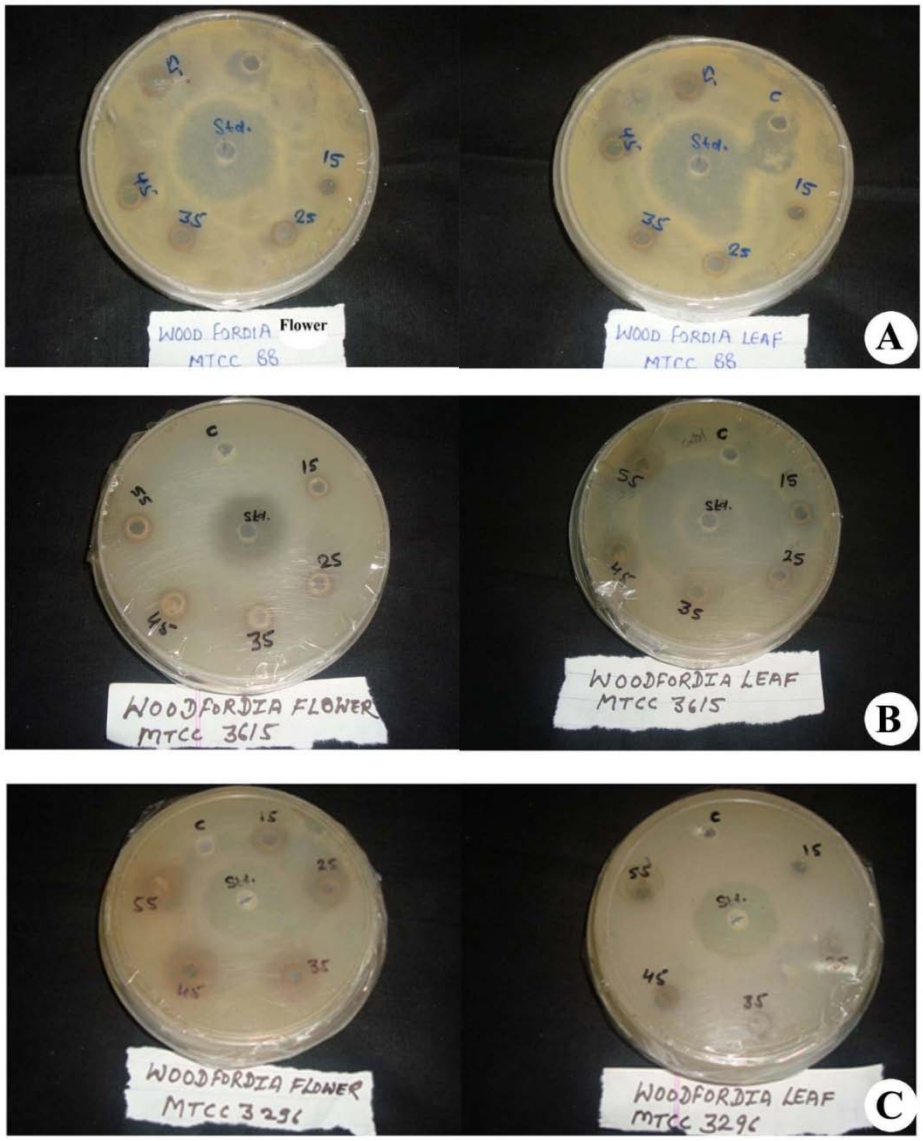


Fig.1.

active principle responsible for such potentials and to develop clinically important therapeutic drugs for mankind.

Previously such studies have been done in several medicinal plants [14]. Hence, an attempt has been made to identify the antimicrobial activity of leaf and flower extracts of *W.fruticosa* against six clinically important microorganisms (four bacteria – two Gram positive, two Gram negative bacteria and two fungi). The results of the antimicrobial activity are presented in Table 1 (Fig. – 1 and 2). Antimicrobial screening of methanolic extract from leaf and flower parts of *Woodfordia fruticosa* revealed that the flower parts of the plant showed better antimicrobial activity against micro - organisms as compared to leaf part. Zone of inhibition increased along with increasing extract concentration. Maximum Zone of inhibition was observed in the flower extracts against *Staphylococcus aureus* (22.4

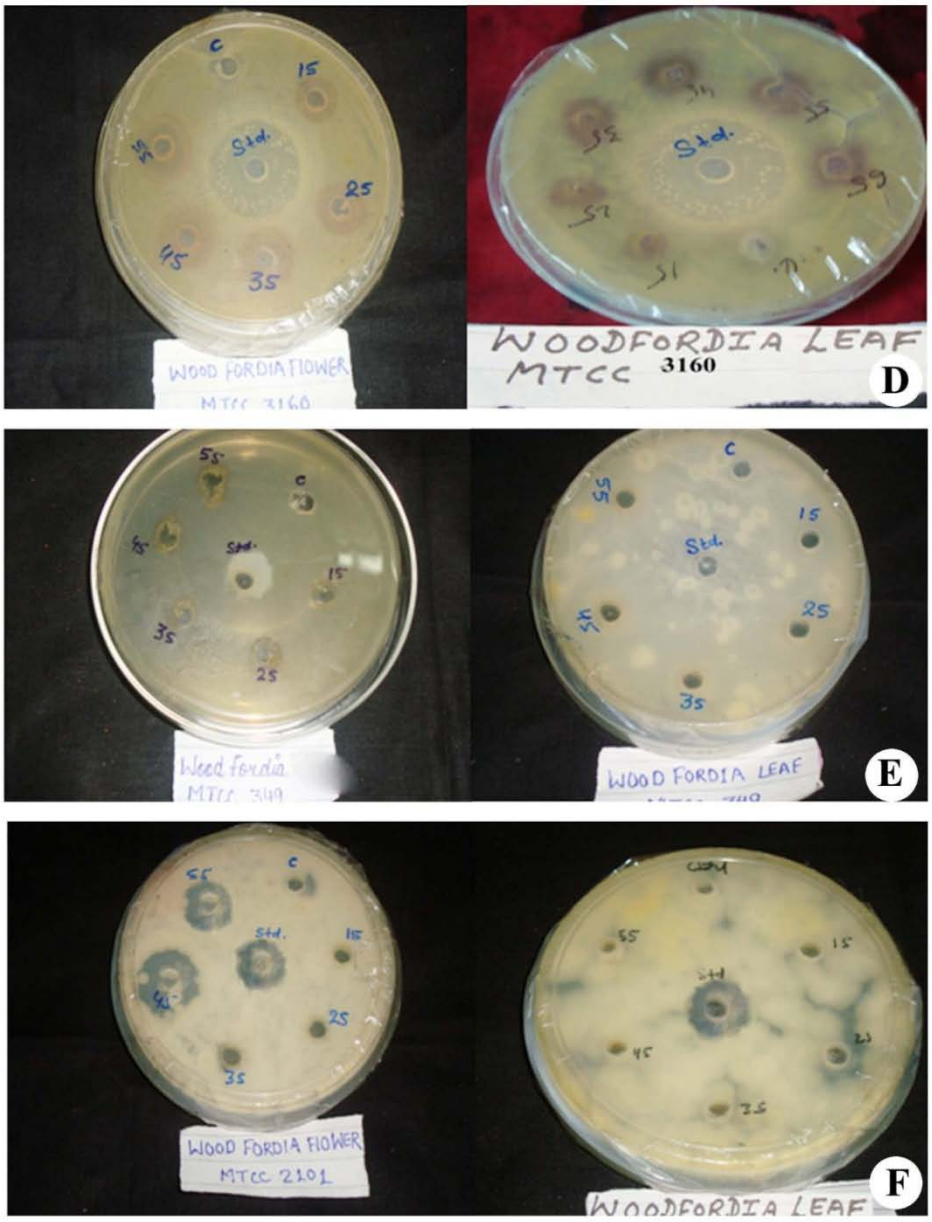
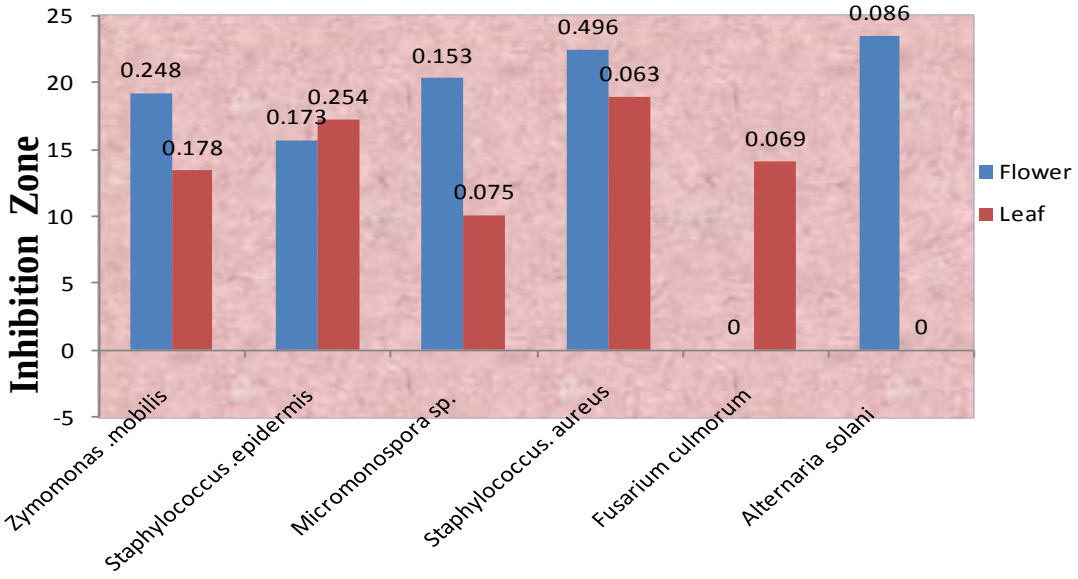


Fig.2.

± 0.86 mm) (Table 1) (Fig. 2. D) amongst the bacteria species and against *Alternaria solani* (23.5 ± 0.15 mm) amongst the fungal species. Minimum zone of inhibition was observed in the leaf extract against *Micromonospora* sp. (10.1 ± 0.13 mm) of bacteria (Fig. 1.C) and in fungal species, *Fusarium culmorum* and *Alternaria solani* showed nil activity in flower and leaf extracts respectively (Fig. 2. E & F). Among bacterial pathogens, gram positive bacterial strains were found to be more susceptible than gram negative bacterial strains. This may be attributed to the fact that cell wall in gram positive bacteria consists of a single layer, whereas gram negative cell wall is a multilayered structure bounded by an outer cell membrane.



Antimicrobial screening (Zone of Inhibition) of methanolic extract from leaf and flower parts of *Woodfordia fruticosa*

Fig.3.

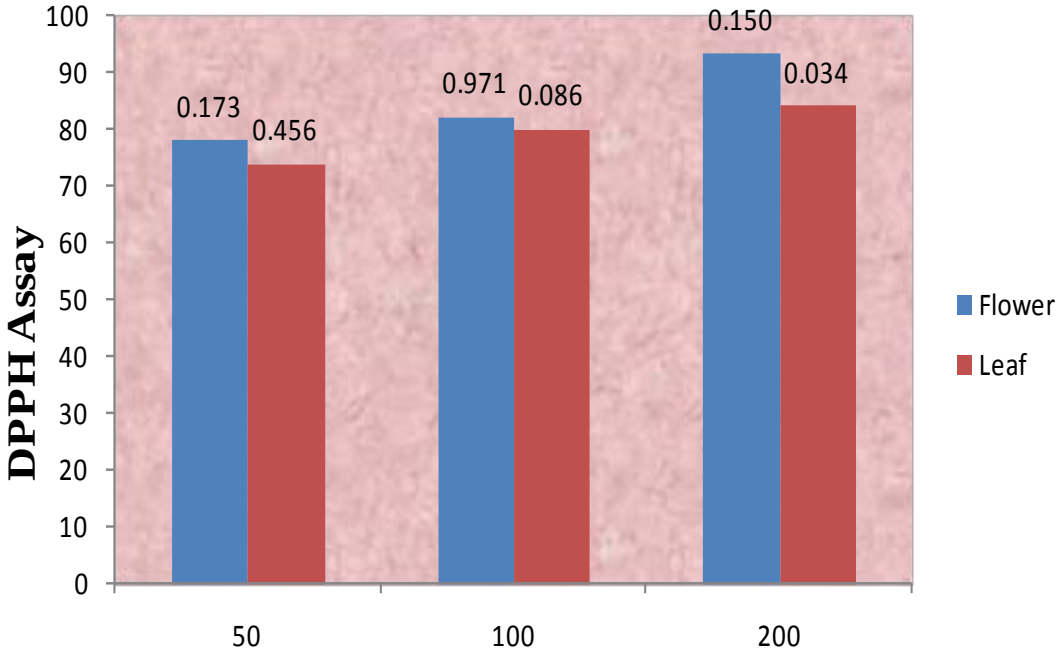


Fig.4.

Similar results were reported earlier also [15-16]. This kind of screening work is very important for the selection of appropriate plant to isolate promising antibacterial agent.

The MIC method was used to further investigate extracts that showed broad spectrum activity against microorganisms. The highest dilution of a plant extract that still retained an inhibitory effect against the growth of microorganisms (absence of zone of inhibition) was reported as the MIC. In this study, methanolic extract of leaf sample showed lowest promising MIC of 8.77 μgml^{-1} in *Fusarium culmorum*. (Table 2). Full results can be seen in Table 1 & 2 and Fig. 3. The minimum inhibitory concentration (MIC) of the flower extract for different micro-organisms ranged between 13.2 and 33.86 μgml^{-1}, while that of the leaf extract ranged between 8.77 and 20.44 µgml-1. Also the MIC of Ampicillin and Flucanazol control ranged between 10.7 and 33.2 µgml-1 (Table 2).

The cidal activities of medicinal plants are due to the active constituents present in them. It is also proved that *Woodfordia fruticosa* contains certain constituents like tannins with significant antibacterial property which enables the extract to overcome the barrier in Gram-negative cell wall [17]. Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism [18]. Tannins play important role such as potent antioxidant. Parekh and Chanda (2007) recorded that crude methanol extracts of *Woodfordia fruticosa* was a good antibacterial agent against all tested microorganisms as it is rich in tannins.

Antioxidant activity: Medicinal plants possess many bioactive compounds including phenolic and polyphenolic compounds which play key function in detoxification of stress induced by free radicals and exhibit antimicrobial activities [19]. All living organisms contain antioxidant metabolites and enzymes which ameliorate various free radical induced damages. Researchers have found a correlation between oxidative damage and the occurrence of diseases [20].

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities. However, if free radicals are produced in excess amount they can be destructive leading to inflammation, ischemia, lung damage and other degenerative diseases. Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases.

A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free-radical scavangers [21-23]. Many reports suggest that plants which are having more phenolic content show good antioxidant activity that is there is a direct correlation between total phenol content and antioxidant activity [24]. The antioxidant capacity possessed by phenolic compounds is mainly due to their redox properties, which permit them to act as reducing agents, hydrogen donors, singlet oxygen quenchers

or metal chelators.

Recently, the DPPH radical scavenging assay has become popular in natural antioxidant studies because of its simplicity and high sensitivity. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH (a stable and commercially available organic nitrogen radical) in test samples. DPPH is widely used to evaluate the free radical scavenging effects of various antioxidant substances and polyhydroxy aromatic compounds. DPPH is a relatively stable free radical.

In this present study, the antioxidant activity of the methanolic extracts of flower and leaf of *Woodfordia fructicosa* were investigated by using DPPH scavenging assay and reducing power of the extract. Flowers extracts of *Woodfordia fructicosa* showed profound and significant percentage (%) DPPH scavenging activity (93.48 ± 0.26) at 200 µg/ml concentration followed by leaves (84.17 ± 0.06) at 200 µg/ml concentration. Moreover, the methanol extract of flower showed prominent activity, which was higher than that of reference antioxidant at all tested concentrations. Results of the activity are presented in Table 3 and Figure 4.

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. This study has similarity with previous investigation.[25] In conclusion, aqueous/methanol extract of *Woodfordia fruticosa* flower was the highest radical scavenger. These results are in agreement with the previous studies which showed that methanol fraction of *Woodfordia fruticosa* flower has highest radical scavenging property [26]. Methanol was found to be a good solvent system for the extraction of the total phenolic compounds. Thus the phenolic and polyphenolic compounds are natural antioxidants which enhance the free radical scavenging activity. While Galvez *et al*., (2005)[27] showed that there is a correlation between antioxidant capacity and phenolic content; however, Kahkonen *et al*., (1999)[28] stated that it is not necessary to correlate antioxidant activity with the high amounts of phenolics.

References

1. Al-Bakri AG and Afifi FU 2007, Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration**.** *Journal of Microbiological Methods* **68** 19-25.
2. Cowan MM 1999, Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**(4) 564-582.
3. Vivek MN, Sachidananda Swamy HC, Manasa M, Pallavi S, Yashoda Kambar, Asha MM, Chaithra M, Prashith Kekuda TR, Mallikarjun N and Onkarappa R 2013, Antimicrobial and Antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima, Delonix regia and Peltaphorum ferrugineum. Journal of Applied Pharmaceutical Science* **3** (08) 064-071.
4. Adesegun SA, Fajana A, Orabueze CI and Coker HAB 2009, Evaluation of antioxidant properties of *Phaulopsis fascisepala*. *Evid. `based Complement. Altern. Med.* **6** 227-231

5. Finkel T and Holbrook NJ 2000, Oxidants, oxidative stress and the biology of ageing. *Nature* **408** 239–247.
6. Thakur RS, Puri HS and Husain A 1989, Major Medicinal Plants of India. Central Institute of Medicinal and Aromatic Plants, P.O. Bag no. 1, RSM Nagar, Lucknow-226016, India.
7. Grover N and Patni V 2013, Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L.) Kurz. Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences* **5**(4) 291-295.
8. Chadha YR 1976, The wealth of India: A dictionary of Indian raw materials and industrial products—raw materials, vol. X. Publication and information directorate, CSIR, New Delhi. pp 586-687
9. Kroes BH, Van-der-Berg AJJ, Heystra EA, De-Silva KTD and Labadie RP 1990, Fermentation in traditional medicine: the impact of *Woodfordia fruticosa* flowers on the immunomodulatory activity and the alcohol and sugar contents of *Nimba arishta* (a portion of fermented *Azadirachta indica* bark). *Planta Medica* **56**(6) 667.
10. Kadota S, Takamori Y, Nyein KN, Kikuchi T, Tanaka K and Ekimoto H 1990, Constituents of the leaves of *Woodfordia ruticosa* Kurz. I. Isolation, structure and proton and carbon-13 nuclear magnetic resonance signal assignments of Woodfruticosin (Woodfordin C), an inhibitor of deoxyribonucleic acid topoisomerase II. *Chemical and Pharmaceutical Bulletin (Tokyo)* **38** 2687–2697.
11. Kuramochi-Motegi A, Kuramochi H, Kobayashi F, Ekimoto H, Takahashi K, Kadota S, Takamori Y and Kikuchi T 1992, Woodfruticosin (woodfordin C), a new inhibitor of DNA topoisomerase II. Experimental antitumor activity. *Biochemical Pharmacology* **44** 1961–1965.
12. Basri DF and Fan SH 2005, The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian J Pharmacol.* **37**(1) 26-29.
13. Toona L, Kambu K, Ngimbi N, Cimanga K and Vlietinck AJ 1998, Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J. Ethanopharmacol*. **61** 63-71.
14. Senthilkumar PK and Reetha D 2009, Screening of antimicrobial properties of certain Indian medicinal plants. *Journal of Phytology* **1**(3) 193–198.
15. Shahwar D and Muhammad AR 2009, *In vitro* antibacterial activity of extracts of *Mimusops elengi* against gram positive and gram negative bacteria. *Afr. J. Microbiol. Res.* **3** 458-462.
16. Chanda S and Baravalia Y 2010, Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms. *Afr. J. Biotechnol*. **9** 3210-3217.
17. Parekh J and Chanda S 2007, *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. flower (Lythraceae). *Brazilian Journal of Microbiology* **38** 204-207.

18. Geidam YA, Ambali AG, and Onyeyli PA 2007, Preliminary phytochemical and antibacterial evaluation of crude aqueous extracts of *Psidium guajava* L. leaf. *J Aplied Sci*. **7** (4) 511-14.
19. Hara-Kudo Y, Kobayashi A, Sugita-Konishi Y, Kondo K 2004, Antibacterial activity of plants used in cooking for aroma and taste. *J. Food Prot*. **67** 2820-2824.
20. Halliwell B and Gutteridge JMC (1999), Oxidative stress. In: Halliwell B, Gutteridge JMC (eds) free radicals in biology and medicine. New York: Oxford university press, pp. 246–350.
21. Abah, SE and Abah G 2010, Antimicrobial and antioxidant potentials of *Agaricus bisporus*. *Advances in Biological Research* **4** (5) 277-282.
22. Manjula M, Kumuda KV, Anitha S, Shashidhara S 2011, Antioxidant and antimicrobial activities of various extracts of *Artabotrys hexapetalus* flowers. *Pharma Science Monitor* **2**(3) 42- 50.
23. Meziti A, Meziti H, Boudiaf K, Mustapha B and Bouriche H 2012, Polyphenolic profile and antioxidant activities of *Nigella sativa* seed extracts *in vitro* and *in vivo. World Academy of Science, Engineering and Technology* **64** 24-32.
24. Salazar R, Pozos M.E, Cordero P, Perez J, Salinas MC and Waksman N 2008, Determination of the antioxidant activity of plants from Northeast Mexico. *Pharm. Biol*. **46** 166-170.
25. Ara N and Nur H 2009, *In vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. *Research Journal of Medicine and Medical Sciences* **4**(1) 107-110.
26. BhattLR and SeungHB 2005, Radica l scavenging activity of extracts and various fractions of *Woodfordia fruti cosa*. *Journal of Cosmetics and Pub lic Health* **1**(2) 68-70.
27. Galvez M, Martin- Cordero C, Houghton PJ and Ayuso MJ 2005, Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem.* **53** 1927-1933.
28. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen M 1999, Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*. **47** 3954-3962.