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Phytochemical and Antimicrobial Properties of Medicinal Plant Crude Extract, Woodfordia fruticosa Linn. From Marathwada, Maharashtra State

Mangesh S. Kharate[1], Narayanb. Pandhure[2]
[1,2]*Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, (M.S.) India*

Abstract: ***The Indian traditional medicinal plant, Woodfordia fruticosa was assessed to determined preliminary biochemical screening as well as the antimicrobial effect of various crude extracts, against pathogenic microorganisms by agar well diffusion method. In preliminary biochemical screening, the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils; as well as chloroform extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. But in hexane extract contains only alkaloids, terpenoids and carbohydrates. The crude extracts show zone of inhibition in the order of methanol> acetone> chloroform> hexane against all test microorganisms.***

Keywords: Phytochemical properties, Antimicrobial activity, Woodfordia fruticosa.

I. INTRODUCTION

Indian traditional medicine, and Indian folklore medicine for several hundred plants. Medicinal plants or their parts are used as source of herbal preparations for the treatment of various diseases based on the experience passed from generation to generation. In order to promote the use of herbal medicines and the determination of their potentials, the studies of medicinal plants should be more intensified. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., while secondary phytochemical as alkaloids to terpenoids and acetogenins to different phenols. The qualitative and quantitative distribution of these metabolites differs from plant to plant and part to part. Alkaloids found in low concentrations relative to the phenolic compounds are offset by their high biological potency in vegetative tissues. Besides this, alkaloids are found in higher concentration in storage tissues (roots, fruits and seeds) as compared to the green leaves [1].Alkaloids and glycosides are complex chemical substances and are distributed in large varieties of the plants throughout the plant kingdom. Most of the secondary metabolites possess medicinal properties. So it is necessary to evaluate the preliminary phytochemical constituents of the selected plant for the further studies. Herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignins, essential oil, monoterpenes, carotenoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes. Secondary metabolites are known to play vital role in management of various diseases.

Woodfordia fruticosa belongs to the family Lythraceae has been traditionally used in India for the treatment of various diseases. All parts of the plant possess valuable medicinal properties but flowers are of maximum demand. Certain compounds were isolated from Woodfordia fruticosa flowers, which include ellagic acid [2], flavonoids [3] and tannins [4, 5, 6, 7, 8 and 9]. Preliminary studies indicated the antioxidant property exhibited by Woodfordia fruticosa flowers [10].

But no detailed investigations were carried out. In view of these the present work undertaken to evaluate the in vitro antimicrobial activity, and to identify the phytochemical constituents of Woodfordia fruticosa.

However, the bioactive potential of compounds from Indian traditional medicinal plants has been little studied, especially in Marathwada region, Maharashtra state.

Therefore, the present study report the phytochemicals and antimicrobial potential of traditional medicinal plant, Woodfordia fruticosa collected from Marathwada region.

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II. MATERIALS AND METHODS

A. *Collection of sample & preparation of crude extract*

The traditional medicinal plant, Woodfordia fruticosa was collected from different sampling stations of from Marathwada region (N $18^0$44'27.81" E $77^0$42'49.53"), Maharashtra, India. The leaf material was collected by an eco-friendly. Identified leaf samples were incised out and (Approx. 100 gm) were washed with tap water, air dried and chopped into small size and extracted with 1000 ml (1:10) methanol, acetone, chloroform and hexane for about 7 days. Then extract was filtered through Whatmann paper No.1 and solvent was removed by rotary vacuum evaporator machine (Buchi type-Superfit, Bangalore) under reduced pressure so as to get the condensed crude sponge extract. The concentrated extract was used for further study.

B. *Preliminary phytochemical screening Woodfordia fruticosa-*

The preliminary phytochemical analysis was carried out using following methods [11, 12]. The leafcrude extracts were qualitatively analyzed for the presence of various biologically active compounds.

1) *Detection of alkaloids*

a) *Mayer's Test:* Extracts were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) *Wagner's Test:* Extracts were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) *Dragendroff's Test:* Extracts were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

d) *Hager's Test:* Extracts were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2) *Detection of glycosides: Legal's Test:* Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

3) *Detection of tannins*

a) *Gelatin Test:* To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

b) *Ferric Chloride Test:* With 1% ferric chloride solution the extract gives blue, green, or brownish green colour indicating the presence of tannins.

4) *Detection of flavonoids*

a) *Alkaline Reagent Test:* Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) *Lead acetate Test:* Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

c) *Shinoda Test:* 2-3 ml of extract, a piece of magnesium ribbon and 1 ml of conc. hydrochloric acid was added. Pink or red coloration of the solution indicates the presence of flavonoids.

d) *Zinc Hydrochloride Test:* To the test solution, add a mixture of zinc dust and conc. Hydrochloric acid. It gives red colour after few minutes.

5) *Detection of proteins and amino acids*

a) *Xanthoproteic Test:* The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

b) *Ninhydrin Test:* To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

6) *Detection of Saponins: Foam Test:* 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of Saponins.

7) *Detection of sterols and terpenoids:* Salkowski's*Test:* Extracts were treated with few drops of conc. sulphuric acid, red colour at the lower layer indicates presence of steroids and formation of yellow colour at the lower layer indicates the presence of Terpenoids.

8) *Detection of carbohydrates*

a) *Molisch's Test:* Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

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b) *Benedict's Test:* Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
c) *Fehling's Test:* Filtrates were hydrolysed with dilutedHCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.
d) *Selwanoffs Test:* One half ml of a sample solution is placed in a test tube. 2 ml of selwinoffs reagent (a solution of resorcinol and HCl) is added. The solution is then heated in a boiling water bath for two minutes. A positive test is indicated by the formation of a red product.
e) *Camnelisation Test:* 1 ml extract were treated with strong sulphuric acid gives a burning sugar smell. This indicates the presence of carbohydrates.
9) *Fats and Fixed Oils: Stain Test:* Small amount of extract were pressed between two filter papers. An oily stain on filter paper indictes the presence of fixed oil.
10) *Antibacterial activity of Woodfordia fruticosa:*) were used as positive control. The plates were incubated at 37°C for 24 hrs. The growth of bacteria around each well was observed carefully and the diameter of the zone of inhibition around each well was measured using a Hi-media zone reader. antibacterial assays were performed by agar well diffusion method is widely used to evaluate the antimicrobial activity of crude extracts [13, 14]. Escherichia coli, Salmonella typhi, (Gram negative bacteria) Bacillus subtilis, Staphylococcus aureus, (Gram positive bacteria) strains were used as test organisms. All bacteria were stored at -20°C until use. Cells were grown at 3°Cin Muller Hinton broth to an Optical density 420 = 1.9 (approx. 105 CFU/ml), and were transfer to Muller Hinton agar. Broth cultures were swabbed onto agar medium to achieve a lawn of confluent bacterial growth separately for each strain. A sterile stainless steel borer (6 mm) was used to make well in the medium. Five wells were bored in each plate. The leaf crude extract (100µg/ml) was loaded in to the well and to find out the inhibitory potential. Triplicate plates were maintained for each test. Discs of Streptomycin (25µg/ml
11) *Antifungal activity of Woodfordia fruticosa:* Assays were performed by agar well diffusion method. The crude extracts were tested against Aspergillusniger, Trichodermaharzianum, Alternariaburnsii and Fusariumoxysporum. The fungal cultures were maintained in 0.2% Sabouraud dextrose broth; each fungal inoculum was applied on plate and evenly spread on Sabouraud dextrose agar using a sterile cotton swab. Discs of the Fluconazole were used as the positive control. The plant leaf crude extract (100µg /ml) was loaded in to the well and to find out the inhibitory potential. The plates were incubated at 28°Cfor 48 hrs.

III. RESULTS

The table 1 shows the results of preliminary phytochemical activity of leaf crude extract of traditional medicinal plant, Woodfordia fruticosa; the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils; as well as chloroform extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. But in hexane extract contains only few biologically active compounds like, alkaloids, terpenoids and carbohydrates. crude methanol, acetone, chloroform and hexane extracts oftraditional medicinal plant, Woodfordia fruticosa were used to investigate the antimicrobial activity against four human pathogenic bacteria as well asfour plant pathogenic fungal species; and the preliminary biochemical screening.Fig. 1 shows result of in vitro testing of sponge extracts against pathogenic bacteria.And the fig. 2 shows results of sponge crude extract against plant pathogenic fungal species. Inhibition zones of extracts against the specific test organisms were measured in mm. The extract restricted the growth of pathogens on the media around wells. The inhibition zone shows of crude methanol, acetone, chloroform and hexane extract in the order of methanol> acetone> chloroform> hexane against all test microorganisms.The maximum inhibition zone (5-7mm) was observed in methanol crude extract against Aspergillusniger, Trichodermaharzianum, Alternariaburnsii and Fusariumoxysporum as well as Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus; and acetone extract shows inhibition against Aspergillusniger, Trichodermaharzianum and Fusariumoxysporum. The minimum inhibition zone (1-3 mm) was noticed in chloroform and hexaneextract against all four pathogenic bacterial strains as well as chloroform show minimum inhibition against hexane against Aspergillusniger, Trichodermaharzianum and Fusariumoxysporum.

IV. DISCUSSION

Plants are the potential sources of natural antioxidants, e.g., carotenoids, flavonoids, isoflavonoids, alkaloids, tannins, phenolic diterpenes and phenolic acids [15]. Thousands of phytochemicals have been identified in plants, yet there are still many that have not been identified. In the present investigation, the identified class of phytochemicals such as flavonoids, alkaloids, tannins, phenolic compounds, saponins and glycosides indicates that the methanolic extract of Woodfordia fruticosa is a rich source of natural

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antioxidants. The preliminary phytochemical tests of crude extract of Woodfordia fruticosa shows the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils.

And chloroform extract contains alkaloids, flavonoids, sterol and terpenoids, carbohydrates, fats and fixed oils. The hexane extract contains only alkaloids, terpenoids and carbohydrates.Thus it is hypothesized that the extract may capable to inhibit the oxidative damage, cancer cell proliferation, regulate inflammatory and immune response, and protect against lipid oxidation. So extract of Woodfordia fruticosais identified as a rich source of phenolics, alkaloids and flavonoids compared to other extracts. Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, ant mutagenic, anticarcinogenic and free radical scavenging activities. Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Alkaloids shows antioxidant and cytotoxic property and their effects on human nutrition and health care are considerable [16]. Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity via the inhibition of lipid peroxidation [17], which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, and anticancer activities. Flavonoids, one of the most diverse and widespread groups of natural compounds, are also probably the most natural polyphenolics capable of exhibiting in vitro antioxidant activities. The results obtained in the present study shown that extract of Woodfordia fruticosacan effectively scavenge reactive oxygen species including hydroxyl radical as well as other free radicals under in vitro conditions. In the present study, methanolic extracts of leaf extract of Woodfordia fruticosa exhibited potent in vitro antimicrobial activity. In the present study, in vitro testing of sponge extracts against pathogens, the inhibition zone shows of crude methanol, acetone, chloroform and hexane extract in the order of methanol> acetone> chloroform> hexane against all test microorganisms. The crude leaf extracts of Woodfordia fruticosawere tested for antimicrobial activities against four bacterial cultures such as, Escherichia coli, Salmonella typhi, Bacillus subtilis and Staphylococcus aureus. result of phytochemical analysis of leaf extract of Woodfordia fruticosa indicates that it is a potential source of phytochemicals with antioxidant and anticancer properties. However, further studies are required to establish its in vivo antioxidant and anticancer activities particularly its chemopreventive efficacy against livercancer. The identified class of components in single or in combination with other components present in the extract might be responsible for the antioxidant activity of the leaf extract of Woodfordia fruticosa. In present work, the crude methanol, acetone, chloroform and hexane extracts of Woodfordia fruticosawere used to investigate the antimicrobial assay against pathogens. The methanol extract shows maximum antimicrobial activity against test microorganisms. Antimicrobial activity against at least one of the test strains, 50% of them showed antibacterial activity while only 20% exhibited antifungal activity.

V. CONCLUSION

The broad spectrum antimicrobial activity of Indian traditional medicinal plants seemed to be due to the presence of alkaloids, tannins, flavonoids and proteins and amino acids, steroids detected in the bioactive fractions. This work confirms the hypothesis on the richness as well as chemical gradient of plant leaf and probably is the first report on the antimicrobial activity of Indian traditional medicinal plant, Woodfordia fruticosa from Marathwada region, Maharashtra, India, to the best of our knowledge. Further research also needs to purify and characterize the secondary metabolites from the Woodfordia fruticosa for the valuable source of novel substances for future discoveries in pharmaceutical science.

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		Extractants			
Phytochemicals		Methanol	Acetone	Chloroform	Hexane
Alkaloids	Mayer's Test	++	+	-	-
	Dragendrorff's Test	+	+	-	-
	Wagner's Test	++	++	+	+
	Hager's Test	++	+	-	-
Glycosides	Legal's Test	+	++	-	-
Tannins	Gelatin Test	+	-	-	-
	Ferric Chloride Test	-	-	-	-
	Shinoda Test	-	-	-	-
	Zinc Hydrochloride Test	-	-	-	-
	Lead Acetate Test	++	++	++	-
Flavonoids	Alkaline Reagent Test	-	-	-	-
Proteins and Amino acids	Xanthoproteic Test	-	-	-	-
	Ninhydrin Test	++	++	-	-
Sterols and Terpenoids	Salkowski Test	++	++	+	+
Carbohydrates	Molisch's Test	+	++	+	+
	Benedict's Test	-	+	-	-
	Camnelisation Test	++	++	+	+
	Selwinoff's Test	-	++	-	-
	Fehling's Test	-	+	-	-
Fats and Fixed Oils	Stain Test	+	++	+	+

(++ Strongly positive; + Positive; - Absent/None)

Table -1:Preliminary phytochemical screening of leaf crude extracts of *Woodfordia fruticosa.*

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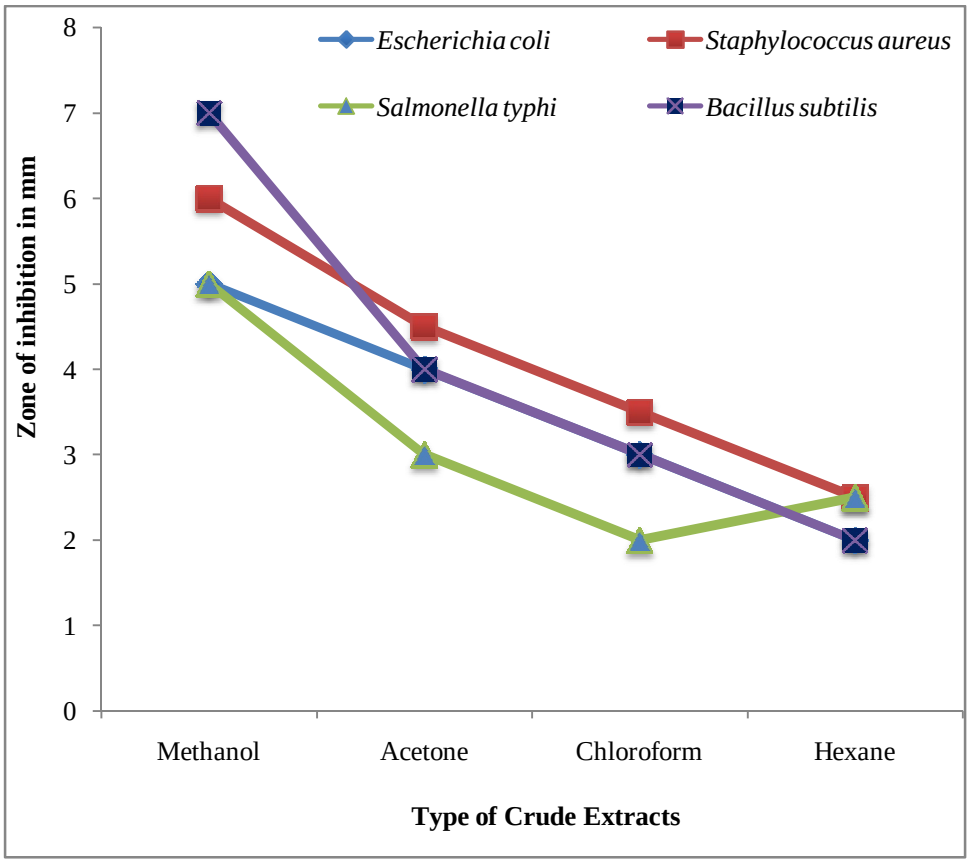


Figure-1:Antibacterial activity of leaf crude extracts of *Woodfordia fruticosa.*

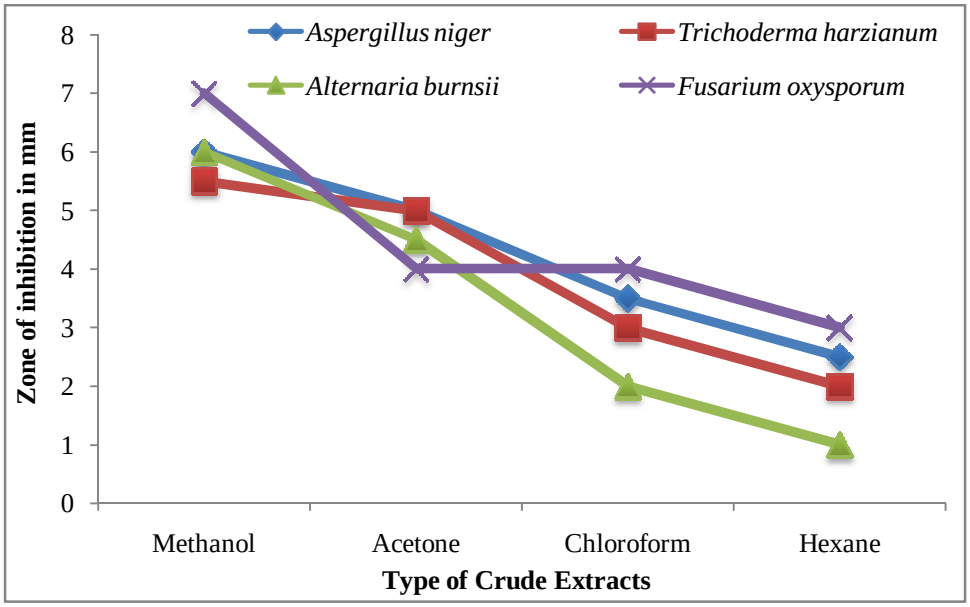


Figure-2:Antifungal activity of leaf crude extractsoWoodforfdiafruticosa.

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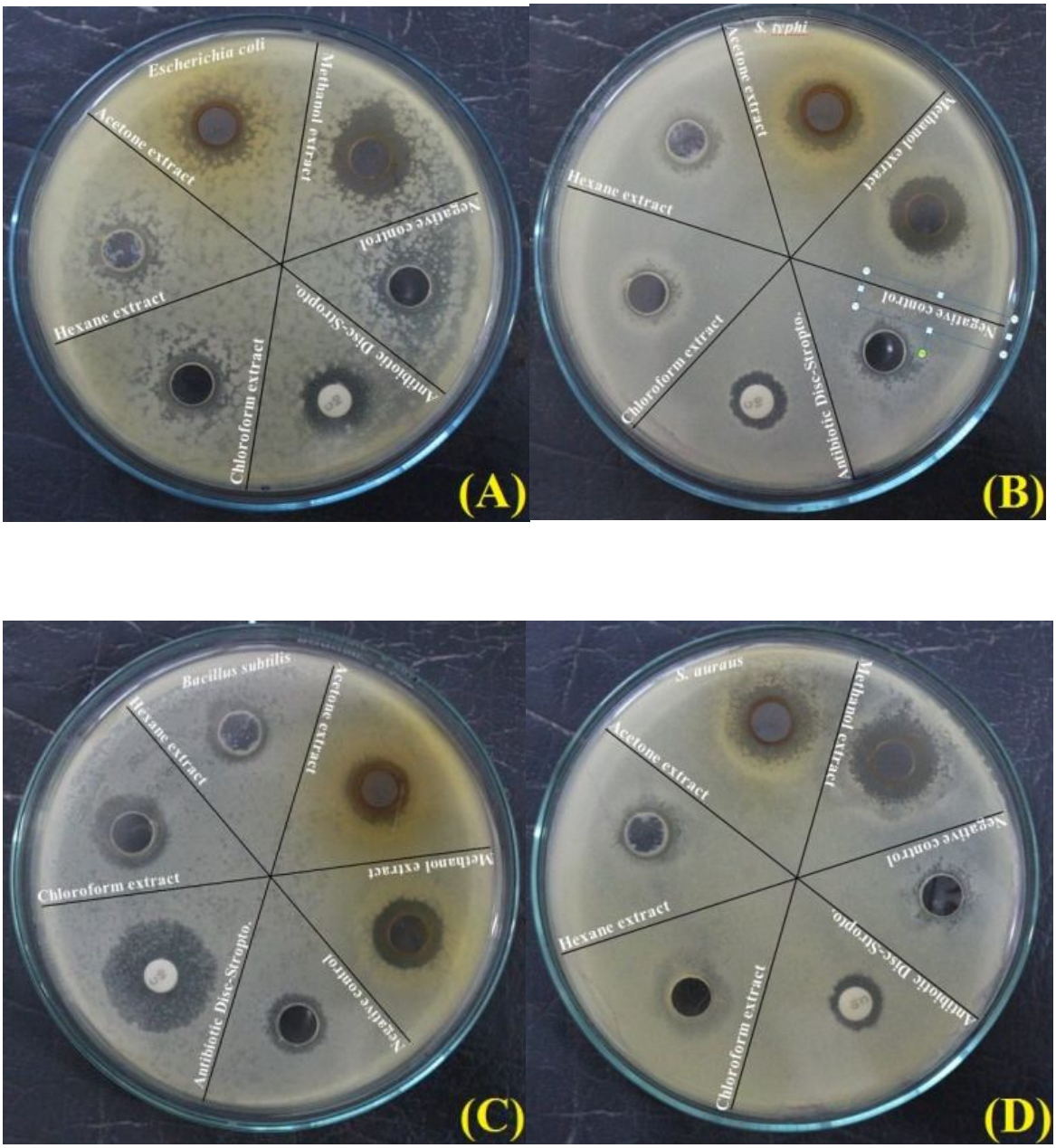


Figure- 1: Showing antibacterial assays of crude extract of Woodfordia fruticosa L. against human pathogens [(A) Escherichia coli, (B) Salmonella typhi, (C) Bacillus subtilis and (D) Staphylococcus aureus].

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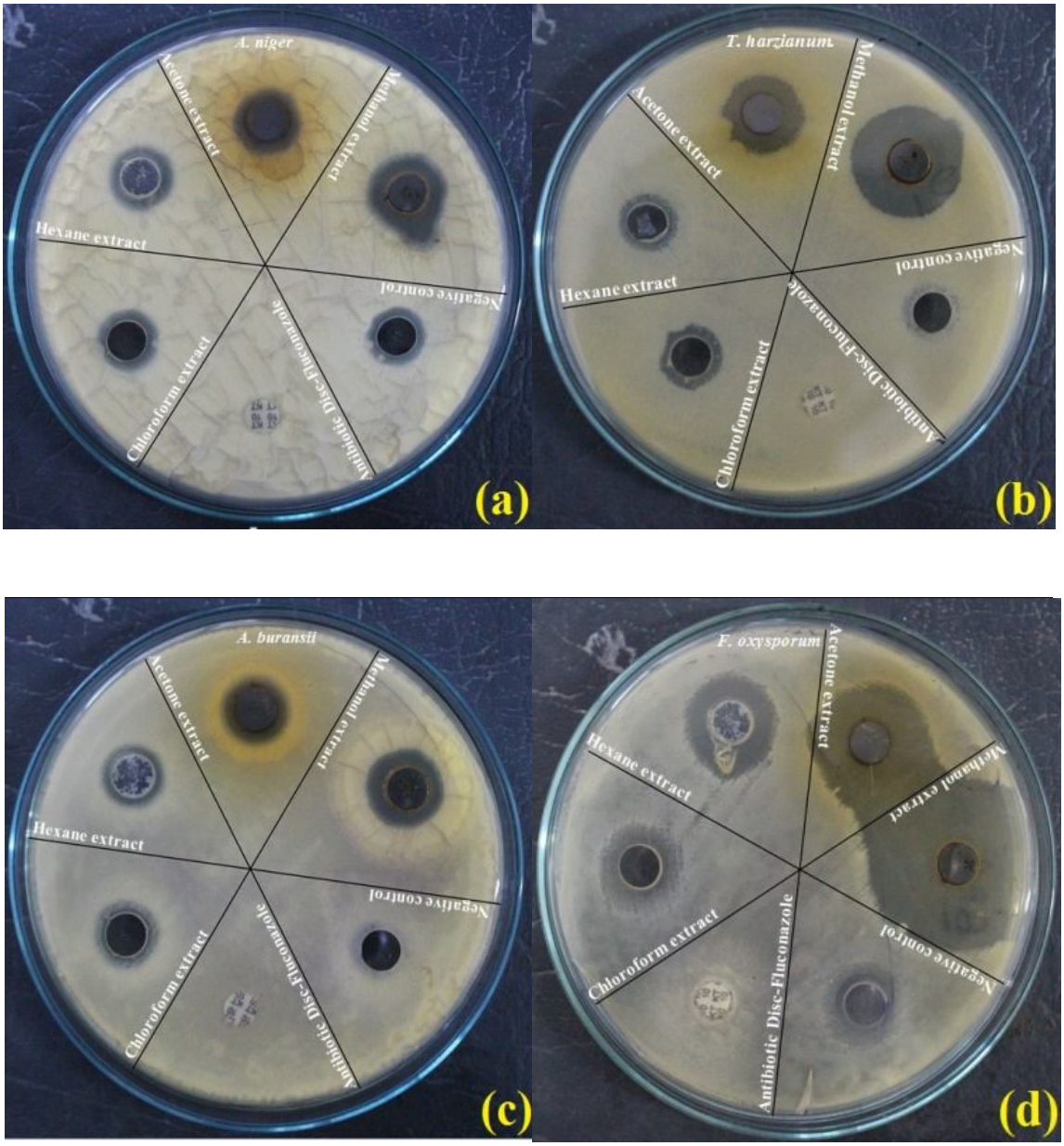
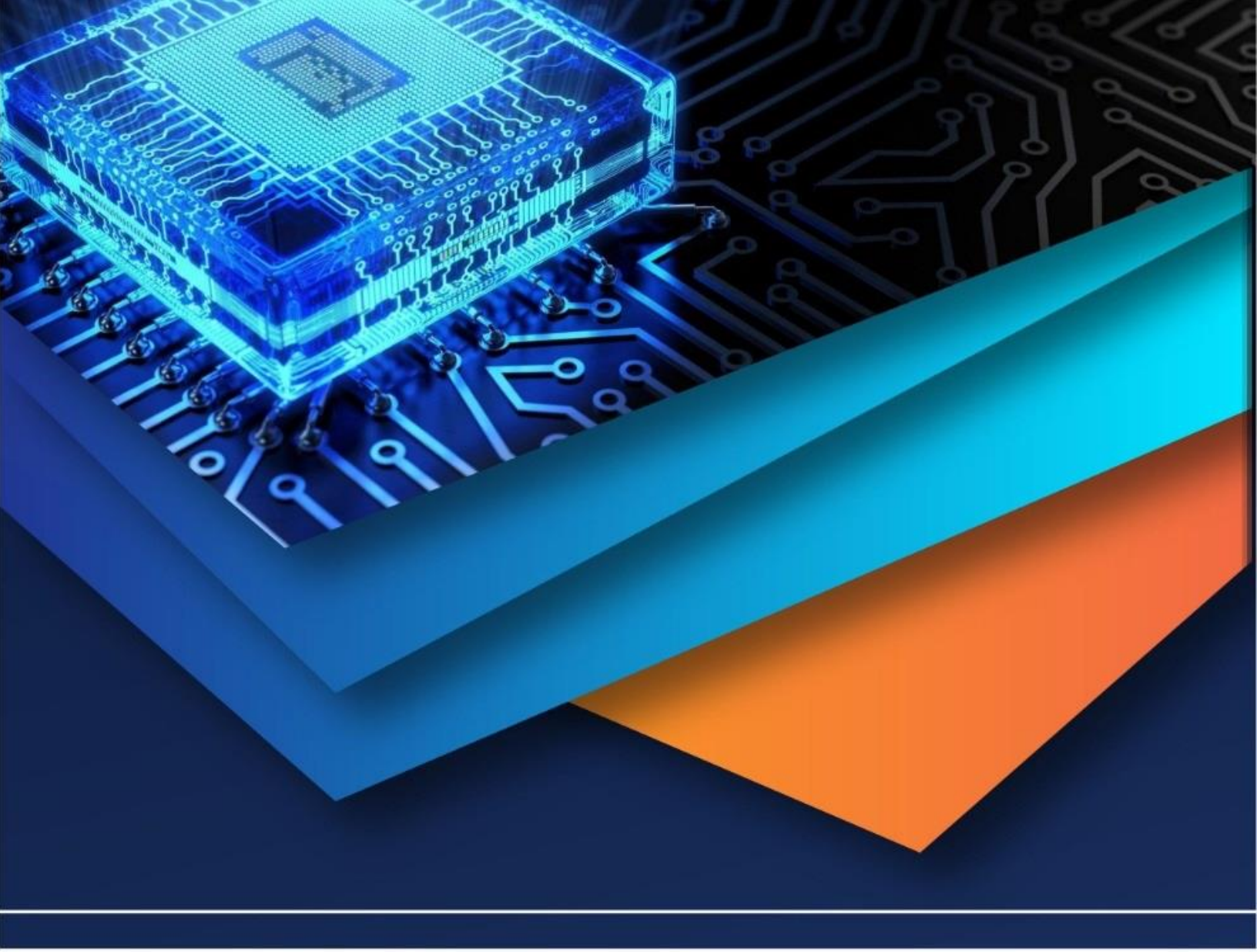


Figure- 2: Showing antifungal assays of all crude extracts of Woodfordia fruticosa L. against plant pathogens [(a) Aspergillusniger, (b) Trichodermaharzianum, (c) Alternariaburnsii and (d) Fusariumoxysporum].

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