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ANTIFUNGAL, ANTIOXIDANT, PHYTOCHEMICAL SCREENING OF *HIBISCUS MOSCHEUTOS* FLOWER EXTRACT AND ACTIVITIES AGAINST SOME HUMAN PATHOGENS

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ABSTRACT

Recent years have witnessed enhanced research work reported on plants and plant products. In this regard, plants with traditional therapeutic usage are being screened more efficiently to be considered as a substitution or as a better alternative agent for Human Pathogens. Antioxidant activity, antibacterial, antifungal properties, colour, phytochemical and FT-IR spectral analysis of flowers belonging to Hibiscus moscheutos was determined. Radical scavenging activity of sample extracts were determined based on the percent inhibition of DPPH and ferric reducing antioxidant power (FRAP) assays. Total phenolics were estimated based on the Folin–Ciocalteu method, while, vanillin–HCl and aluminum chloride methods were employed to estimate total tannins and flavonoids in the sample extracts, respectively. To determine total flavonols and anthocyanin contents, spectrophotometric method was employed. For antibacterial activities, modified agar disk diffusion method was adopted. Color analysis was performed using a colorimeter, while functional groups of compounds were identified using a FTIR-spectrophotometer. In this study, the flower extracts encompass high amount of antioxidant compounds and exhibit significant antioxidant activities, which depended on extraction solvents. Methanol extracts of Hibiscus moscheutos had high total phenolic, total flavonoid and total flavonol content, and showed highest activity for inhibition of DPPH. The colour analysis of Hibiscus moscheutos showed lower chroma and hue angle values. The crude extracts of hexane, dichloromethane, chloroform, ethyl acetate and methanol, extracts with concentration levels at 25, 50, 100, 250, and 500 mg/mL were shown to significantly affect the inhibition of bacterial and fungal selected. Among the crude extracts, ethyl acetate and methanol extract was shown to be the most potent in terms of antifungal activities. The profile of the flower extract observation proved the chemical constituent that the treatment altered the fungal and bacterial morphology, which leads to the organism growth inhibition. For the in vivo bioassay, the pathogen treated with ethyl acetate and methanol extract at 250 and 500 mg/mL showed the higher inhibition value. There were 49 chemical compounds identified in Hibiscus moscheutos methanol extract using GCMS analysis. The top five major compounds were dominated by squalene triterpene (13.17%), phytol (3.18%), Neophytadiene (5.36%), spinasterol acetate (7.41%), and tocopherol acetate (7.41%). Some of these significant compounds possess high antifungal and antibacterial activities. This study proved that Hibiscus moscheutos flowers from the five solvent extract could be useful for inhibiting the selected pathogens which has potential as a natural antifungal and bacterial agent.

KEYWORDS: Antifungal, Antibacterial, Antioxidant, Phytochemical, Hibiscus moscheutos, Human, Pathogens.

INTRODUCTION

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases.[1]

The numerous experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components.[2,3] Traditionally, several plants and their products have been used in foods (as herbs or spices) as a mode of natural preservative, flavouring agent as well as a remedy to treat some of the common ailments in humans. This property of curing is attributed mainly to their antimicrobial activities. Use of natural plant derived

antimicrobials can be highly effective in reducing the dependence on antibiotics, minimize the chances of antibiotic resistance in food borne pathogenic microorganisms as well as help in controlling cross-contaminations by food-borne pathogens.[4] In addition to the antioxidant and antimicrobial activities exhibited by plants or their extracts, they can also be used as natural colorants of foodstuffs; as in most of the cases, they are believed to be safe, and non-toxic to humans.[5,6,7, and 8]

Hibiscus moscheutosis a profusely flowering, perennial, woody ornamental shrub distributed widely in the tropical regions., the herbaceous perennial species of hibiscus native to the United States are all handsomely flowered, but except for *H. coccineus* are rather coarse plants which often seem out of place in restricted or small perennial borders. For this reason they are not encountered as frequently as one might expect from the showiness of the flowers alone. In this case *H. coccineus* was crossed with *H. militaris*, and an individual of this cross was in turn crossed with *H. moscheutos.* Progeny from this cross had flowers varying in size from 10 to 25 cm in diameter, in colours ranging from white through pink to deep red, often with a deeper eye at the centre. A similar hybridization program involving the same three parents was commenced in the early 1950s, and the results were collectively termed 'Avalon Hybrids'. Selected forms of these hybrids were crossed with *H. moscheulos* and *H. grandiflorus* to produce the giant flowering `Southern Belle' strains with blooms up to 30 cm across.

H. moscheutos hybrids are easily grown from seed sown in spring, usually flowering the first season. Fresh seed germinates readily. Selected varieties may be propagated by root division or tip cuttings. Tolerant of frosts, these hardy plants are cultivated over a much wider range of climates.

Of late, many reports are available wherein flowers or their extracts have been shown to exhibit rich biochemical and antimicrobial properties.[9, 10, and 4]

The increasing recognition and importance of bacterial and fungal infections, the difficulties encountered in their treatment and the increase in resistance to antibacterial and antifungal agents have stimulated the search for therapeutic alternatives.[11] The products of plant secondary metabolism have application in folk medicine, fragrance industries, food flavouring and preservation but only in recent years they have started to be recognized for their potential antimicrobial role.[12] Although numerous studies have documented the plant extracts antibacterial and anticandidal effect.[12, 13, 14, 15, 16, 17, and 18] There have been few comprehensive *in vitro* studies of the effects exerted by flower extract on the filamentous fungi, probably due to the difficulties encountered in standardized susceptibility methods for these mycetes.[19, 20, 21, and 22]

In many research centres are conducted intensive *in vitro* studies on the mechanism of action and safety of plant extracts and their individual components. The obtained results encourage to further investigations on the usefulness of the crude extract of the flower in combating a particularly dangerous antibiotic-resistant microorganisms. However, of late, many reports are available wherein flowers or their extracts have been shown to exhibit rich biochemical and antimicrobial properties.[9, 10, and 4]

Based on this, the present study was aimed at evaluating the antimicrobial activities against Gram- positive and Gram-negative bacteria, fungi and access the colour properties as well as identifying the presence of various functional groups chemical profiling of the crude(based on GC-MS and FTIR spectra respectively) in one of the widely distributed ornamental flowering plant Hibiscus moscheutosis (red colour flowers). It is anticipated that results generated from this work will provide a suitable base in the use of these flowers as a natural remedy for antimicrobial effects as well as developing novel drugs.

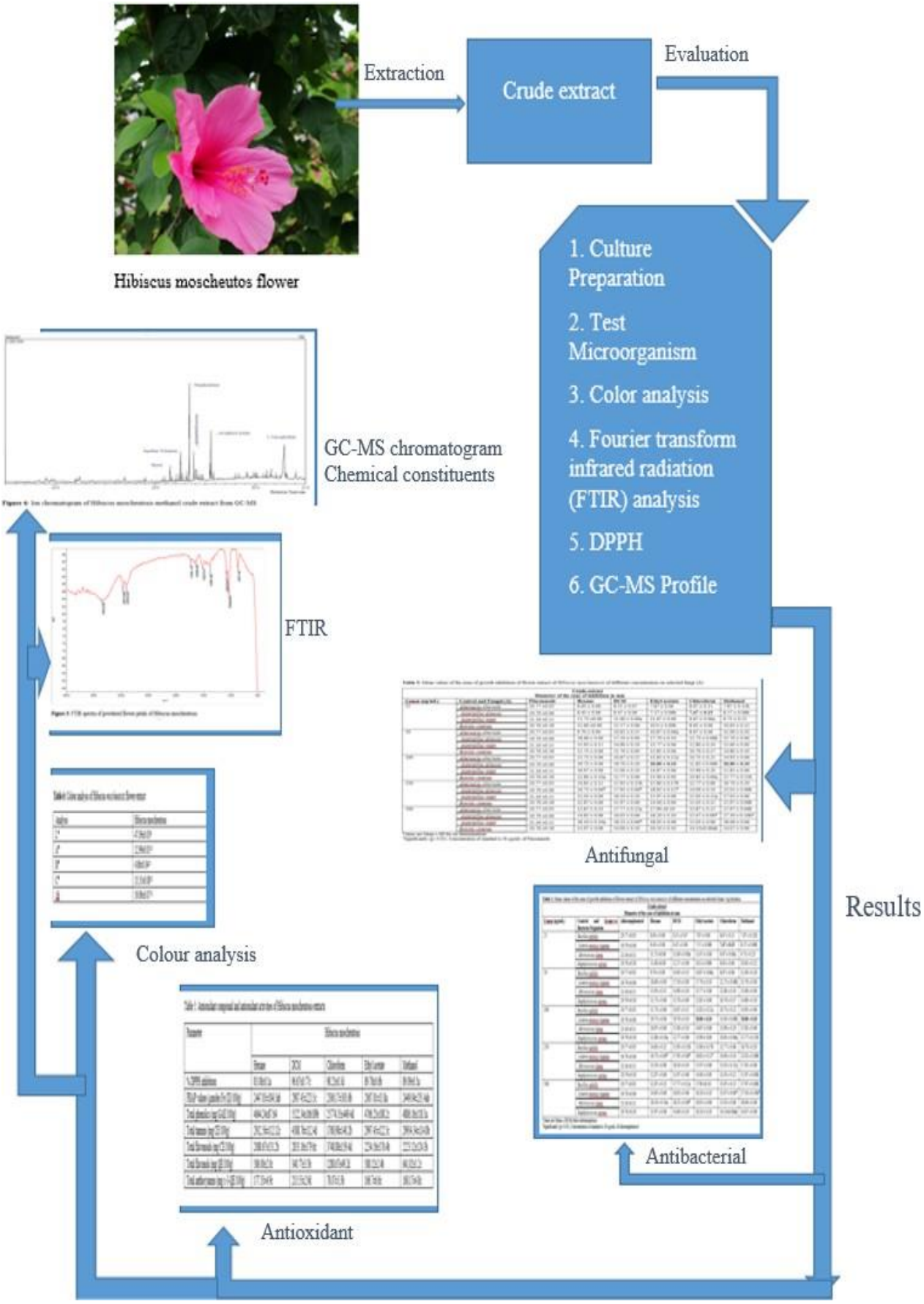

Figure 1: Graphical abstract Antifungal, Antibacterial, antioxidant activity and Phytochemical screening of *Hibiscus moscheutos* flower extract against some Human Pathogens.

1. MATERIALS AND METHODS

1.1 Samples

Fresh flowers of hibiscus (*Hibiscus moscheutosis*) with no apparent physical, insect or microbial damage were collected from the forest reserve garden in Michika local government. The flower petals were carefully removed (without anther, stamen or sepals) and were freeze-dried (freeze dryer Model, LD53, Kingston, New York) for 48 hr. at -50^{0}C. Samples were powdered (mesh size 30), covered with aluminium foil (to avoid exposure to light) and stored at 4^{0}C until analysis (performed within 24 hr.).

Extract preparation

Five different solvent were used for the extraction of the crude extract, Extraction was carried out by the conventional solvent extraction method described by Umaru et al.[23] This was achieved by soaking the ground plant material in solvents in the order of increasing polarity. A total of 500g of the dried and ground powdered sample was extracted using cold soaking method with hexane. The sample were soaked in the hexane with the ratio of 1:3 (sample: hexane) in a 5 litres Erlenmeyer flasks at room temperature for 72 hrs. The resulting hexane solution was then filtered using Whatman filter paper No 4 and the residue was then re-extracted with fresh hexane for another 72 hrs and filtered. Both extracts were combined and evaporated to dryness with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure below 50^{O}C to obtain the hexane crude extract. The residue was re-extracted using similar procedure with dichloromethane, followed by chloroform, ethyl acetate and methanol to obtain respective crude extracts.

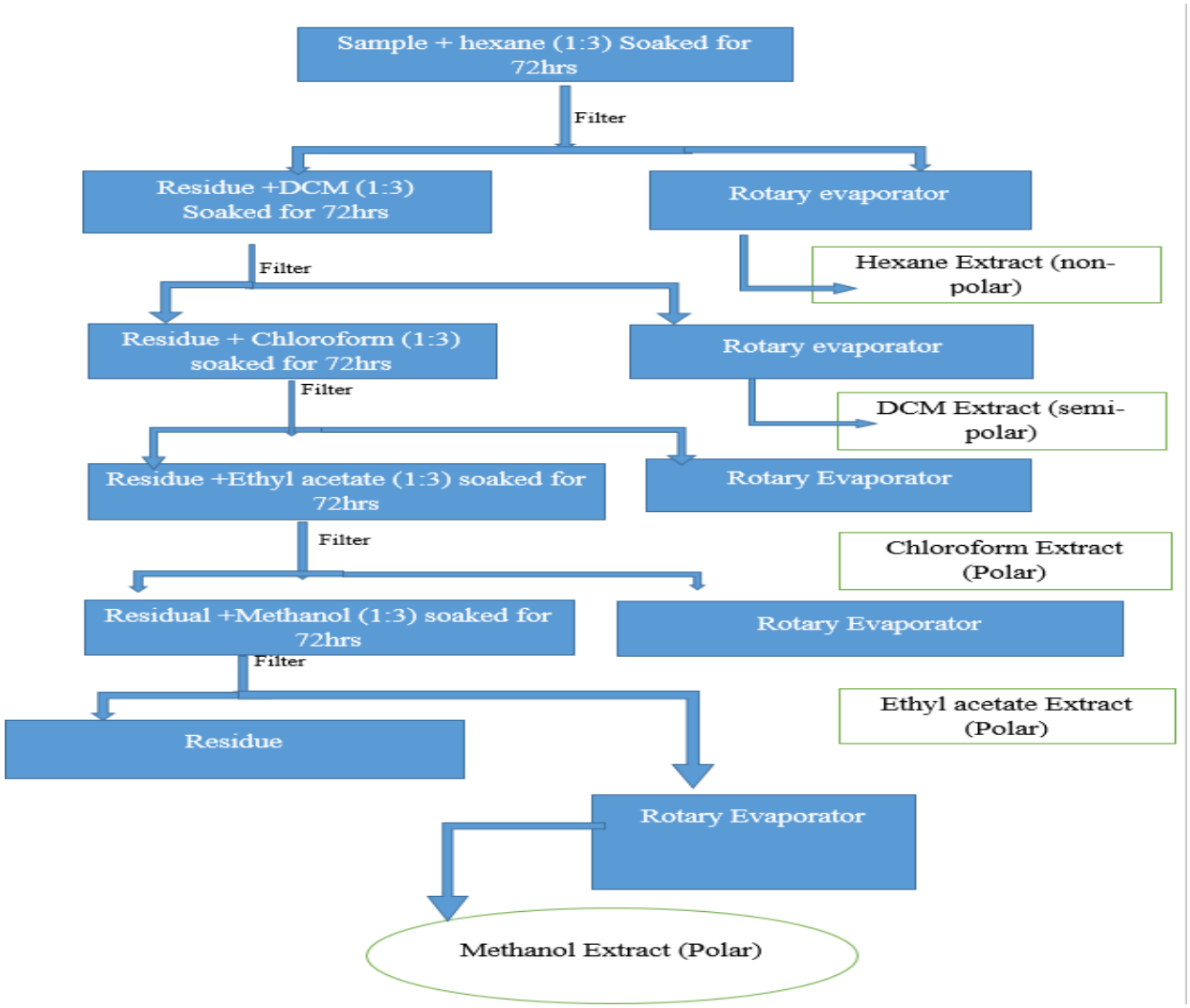

Figure 2: Sequential extraction procedure of *Hibiscus moscheutos* flower.

1.2 Culture preparation

Suspension of tested pathogens (108 CFU/mL) was spread on BHI agar (using sterile-glass hockey stick) to ensure even distribution of the suspension on the agar plate. The agar plates were left to be fully diffused after each application. Based on the modified agar disk diffusion method[24], inhibitory potential of microbial growth was determined. Sterile blank paper disks (Oxoid, England, 6 mm diameter) were impregnated with 50 µl of the extracts (500 mg/mL, 400 mg/mL,

300 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL), left to be dried and gently pressed on the inoculated agar plates. Distilled water and ethanol served as the negative control while chloramphenicol (Oxoid, England, 10 lg/ disk) served as positive control. Agar plates were incubated at 37^0C for 24 hr. Antibacterial activity was determined by measuring the diameter of the clear zones of inhibition.

The antifungal activity of extracts was determined by diffusion method in Petri dishes (10 cm) with a solid medium PDA. On each substrate 4 drops of an aqueous suspension of spores and mycelia fragments was applied, next uniformly distributed on the surface, then they were dried and placed on a paper disk (6 mm) soaked with botanical extract. The measure of the extracts activity was the size of the zone of inhibition of growth colonies (in millimetres) measured after 5 days incubation in 22°C. The experiment was established in 6 replicates for each extract, (500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL. Each repetition consisted of four Petri dishes.

1.3 Test microorganisms

The antibacterial activity of the plant extracts were tested *in vitro* against the Gram-positive bacteria: *Bacillus subtilis, Listeria monocy togenes, Micrococcus luteus, Staphylococcus aureus;* and the Gram negative bacteria: *Escherichia coli, Acinetobacter baumanni, pseudomonas aeruginosa, and Chlamydia trachomatis.* The antifungal activity of plant extracts was studied against eight different microorganisms, including: *Alternaria alternata (Fr.) Keissler, Aspergillus glaucus (L.) Link, Aspergillus niger (Tiegh.), Botritis cinerea (Pers), Cladosporium herbarum (Pers.) Link ex Fr, Fusarium culmorum (Sacc.), Fusarium poae (Peck), Penicillium chrysogenum (Thom),* All microorganisms were obtained from the stock cultures of the Microbiology Laboratory Federal University Wukari.

1.4 Colour analysis

The powdered samples of flowers were subjected to colour analysis using a colorimeter (Minolta, Spectrophotometer CM-3500d, Japan). Powdered samples were placed individually in the specimen cell for measurements. Minolta colour scale was used to measure the lightness, which was indicated by L* value [L* = 0 (black) to L* = 100 (white)]. The, a* and b* values that shift from negative to positive values are an indication of the shift from bluish-green to purplish-red and from blue toyellow, respectively.

1.5 Fourier transform infrared radiation (FTIR) analysis

FTIR spectra were obtained from KBr pellets prepared using 1.0 mg of powdered flower samples. The pellets were analysed in the absorption mode of FTIR and all spectra were recorded from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} using a FTIR spectrophotometer (System 2000, Perkin Elmer, and Wellesley, MD, U.S.A.).

1.6 DPPH radical scavenging activity and ferric reducing antioxidant power assay (FRAP)

The capacity of the flower extracts to scavenge DPPH radicals (2, 2-diphenyl-1-picrylhydrazyl) was measured based on the method described by Umaru et al.[25] The results obtained were expressed as the percentage inhibition of DPPH based on the following formula.

Percent inhibition of DPPH = [(A control _ A sample)/A control] X 100

Where A control is the absorbance of the DPPH solution without sample extract and A sample is the absorbance of the sample with DPPH solution. For FRAP assay, a modified method described by Yin et al.,[26] Was adapted to measure the ability of extracts to reduce ferric ions. Ferrous sulphate solution (concentration ranging from 0.1 to 1µM) was used for preparing the standard calibration curve. FRAP activity was expressed as micromoles of Fe (II)/100 g of dry weight of samples.

1.7 Determination of total phenolic content, tannins

Favonoids and flavonols, the total phenolic content of the flower extracts were determined based on the Folin–Ciocalteu (FC) method.[27] In brief, 400 lL of the sample extract was mixed with 2.0 mL of FC reagent (10 times pre-diluted). Further, after incubation for 5 min at room temperature, 1.6 mL of (7.5%, w/v) sodium carbonate solution was added and the solution was mixed thoroughly and incubated for 60 min at room temperature. Followed by this, absorbance was measured using a UV–visible spectrophotometer (Shimadzu UV-160A, Kyoto, Japan) at 765 nm. A suitable calibration curve was prepared using standard gallic acid solution. All the results were expressed as mg Gallic acid equivalents (GAE) per gram of sample. For total tannins, the vanillin–HCl method was employed.[28] Briefly, 1 mL of the sample extracts was treated with 5 mL of reagent mixture (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1 ratio). The color developed was read after 20 min. at 500 nm using a UV–visible spectrophotometer (Shimadzu UV-160A). Suitable standardcalibration curve was prepared using catechin (20-400 lg/mL) and results were expressed as mg Catechin equivalent (CE) per 100 g dry weight of the samples, respectively. Total flavonoids in the sample extracts were determined using the aluminium chloride method as described in the report of Liu et al.[29] In brief, for 500 mL of the sample extract solution, 2.5 mL of distilled water and sodium nitrite solution (5%, w/v, 150 mL) were added to the mixture. This mixture was maintainedfor 5 min., followed by addition of 300 mL of aluminium chloride (10%, w/v) and again incubated for6 min. Followed by this, 1 mL of sodium hydroxide (1 M) was added and the mixture was dilutedwith 550 mL of distilled water. This solution was mixed vigorously and the absorbance of the mixture was measured immediately at 510 nm using a UV–visible spectrophotometer (Shimadzu UV-160A, Shimadzu

Corporation, Kyoto, Japan). Results of the total flavonoid content were expressed as mg Catechin equivalents (CE) per 100 g of dry weight of the sample. Total flavonols in the sample extracts were evaluated based on the method described by Miliauskas et al.[30] with slight modifications. Briefly, 1 mL of 0.15–0.05 mg/mL quercetin methanol solution with 1 mL of 2% aluminium trichloride and 3 mL of 5% sodium acetate were mixed to obtain a quercetin calibration curve. After 150 min and incubation at 20ºC, the absorption was read at 440 nm using a UV–visible spectrophotometer (Shimadzu UV-160A). This procedure was repeated using 1 mL of the sample extract (1 mg/mL) instead of quercetin solution. Results obtained were expressed as mg Quercetin equivalent (QE) per 100 g dry weight of samples. To determine total anthocyanins, the spectrophotometric method detailed by Abdel-Aal and Hucl[31] was employed. Briefly, anthocyanins were extracted using acidified methanol (methanol and 1M HCl, 85:15, v/v) with a solvent to sample extract ratio of 10:1. This was centrifuged and the absorbance was measured at 525 nm using a UV–visible spectrophotometer (UV-160A, Shimadzu, Japan) against a reagent blank. Cyanidin-3- glucoside (5, 10, 15, 20, and 25 mg/L, r2 = 0.9982) was used to prepare for the standard calibration curve. Total anthocyanin contents in the flower extracts were expressed as mg cyanidin-3-glucoside equivalents per 100 g dry weight of samples.

2. RESULT

In this study, PIRG was measured and calculated to determine the antifungal, antibacterial activity of five types of crude extracts at five different concentrations. As shown in Table 1 –Table 6. The main factors of the crude extracts and the concentration levels significantly inhibited the in vitro growth of the selected pathogen. There was also a highly significant interaction effect between crude extract and concentration levels (CE & CL) on the selected pathogens growth. The GCMS analyses ofthe crude extract led to the identification of 49 chemical constituents in the methanol crude extract of *Hibiscus moscheutos* flower. Determination of total phenolic content, tannins, flavonoids and flavonols Table 5 and colour analysis. FTIR spectra were obtained from KBr pellets prepared using 1.0 mg of powdered flower samples. The functional were as shown in Table.

Table 1: Mean values of the zone of growth inhibition of flower extract of *Hibiscus moscheutosis* ofdifferent concentration on selected Gram +ve bacteria.

Crude extract Diameter of the zone of inhibition in mm							
Concn (µg/mL)	**Control and Gram+ve Bacteria Organism**	**Chloramphenicol**	**Hexane**	**DCM**	**Ethyl acetate**	**Chloroform**	**Methanol**
25	*Bacillus subtilis*	21.73 ±0.03	9.33 ± 0.06	8.54 ± 0.07	9.87 ± 0.06	8.98 ± 0.17	9.87 ± 0.22b
	Listeria monocy togenes	20.79 ±0.06	9.43 ± 0.06	9.47 ± 0.06	9.57 ± 0.06b	**8.47 ± 0.14**	9.57 ± 0.16b
	Micrococcus luteus	22.16 ±0.11	12.73 ±0.06	10.60 ± 0.00a	11.67 ± 0.06	10.67 ± 0.06a	10.73 ± 0.13
	Staphylococcus aureus	21.76 ±0.18	13.60 ±0.00	12.57 ± 0.06	13.0 ± 0.00b	11.63 ± 0.06	11.63 ± 0.13
50	*Bacillus subtilis*	21.77 ±0.03	10.70 ± 0.00	10.63 ± 0.15	11.87 ± 0.06a	10.67 ± 0.06	13.00 ± 0.22
	Listeria monocy togenes	20.79 ±0.06	19.60 ± 0.00	18.50 ± 0.03	18.70 ± 0.10	13.73 ± 0.06b	14.70 ± 0.03
	Micrococcus luteus	22.16 ±0.11	14.53 ± 0.15	15.90 ± 0.17	15.77 ± 0.06	15.80 ± 0.10	15.60 ± 0.05
	Staphylococcus aureus	21.76 ±0.18	15.76 ± 0.06	15.70 ± 0.06	14.83 ± 0.06	14.70 ± 0.17	17.80 ± 0.11
100	*Bacillus subtilis*	21.77 ±0.03	14.73 ± 0.06	16.67 ± 0.16	13.83 ± 0.12a	17.73 ± 0.21	17.93 ± 0.07
	Listeria monocy togenes	22.79 ±0.06	20.73 ± 0.06	19.88 ± 0.11	**21.80 ± 0.10**	16.83 ± 0.06b	**22.60 ± 0.15**
	Micrococcus luteus	22.16 ±0.11	17.97 ± 0.06	16.00 ± 0.12	15.87 ± 0.06	15.90 ± 0.20	16.83 ± 0.23
	Staphylococcus aureus	21.76 ±0.18	15.80 ± 0.10a	14.77 ± 0.06	14.90 ± 0.00	14.83 ± 0.06a	18.77 ± 0.23b
250	*Bacillus subtilis*	21.77 ±0.03	16.83 ± 0.12	15.93 ± 0.12b	16.80 ± 0.17b	16.77 ± 0.66	17.70 ± 0.25
	Listeria monocy togenes	20.79 ±0.06	15.73 ± 0.06*	17.93 ± 0.11*	19.83 ± 0.12*	17.00 ± 0.10	22.03 ± 0.04b
	Micrococcus luteus	20.16 ±0.11	14.34 ± 0.05	18.10 ± 0.14	17.97 ± 0.06	18.03 ± 0.12a	18.03 ± 0.07
	Staphylococcus aureus	21.76 ±0.18	14.86 ± 0.04	13.97 ± 0.12	16.00 ± 0.00	18.03 ± 0.12	16.97 ± 0.08b
500	*Bacillus subtilis*	22.77 ±0.03	13.55 ± 0.25	18.77 ± 0.13a	19.90 ±0.10	17.87 ± 0.15	18.97 ± 0.02b
	Listeria monocy togenes	19.79 ±0.06	15.45 ± 0.07	17.03 ± 0.16	18.20 ± 0.10	19.07 ± 0.06*	23.30 ± 0.13b*
	Micrococcus luteus	22.16 ±0.11	19.30 ± 0.13a	18.23 ± 0.08	19.03 ± 0.06	18.03 ± 0.06	20.06 ± 0.14
	Staphylococcus aureus	21.76 ±0.18	18.57 ± 0.12	19.00 ± 0.14	19.10 ± 0.10	19.13±0.06ab	18.07 ± 0.11

Values are Mean ± SD for three determinations

*Significantly ($p < 0.05$). Concentration of standard is 30 µg/mL of chloramphenicol

Table 2: Mean values of the zone of growth inhibition of flower extract of *Hibiscus moscheutosis* ofdifferent concentration on selected Gram -ve bacteria.

Crude extract Diameter of the zone of inhibition in mm							
Concn (µg/mL)	**Control and Gram–ve Bacteria Organism**	**Chloramphenicol**	**Hexane**	**DCM**	**Ethyl acetate**	**Chloroform**	**Methanol**
25	*Escherichia coli*	22.77 ±0.03	8.44 ± 0.06	8.76 ± 0.07	91.11 ± 0.16	9.17 ± 0.15	9.87 ± 0.132b
	Acinetobacter baumanni	20.79 ±0.06	8.56 ± 0.06	8.33 ± 0.06	9.23 ± 0.12b	**8.78 ± 0.17**	10.57 ± 0.16b
	Pseudomonas aeruginosa	22.16 ±0.11	11.33 ±0.06	11.16 ± 0.04a	11.17 ± 0.26	10.17 ± 0.05a	9.88 ± 0.20
	Chlamydia trachomatis.	21.76 ±0.18	11.40 ±0.00	13.88 ± 0.07	12.10 ± 0.10b	9.12 ± 0.13	11.63 ± 0.22
50	*Escherichia coli*	21.77 ±0.03	10.15 ± 0.00	11.63 ± 0.15	11.17 ± 0.16a	9.67 ± 0.16	14.00 ± 0.10
	Acinetobacter baumanni	20.73 ±0.06	19.60 ± 0.00	15.17 ± 0.02	15.10 ± 0.12	14.73 ± 0.11b	15.70 ± 0.10
	Pseudomonas aeruginosa	21.15 ±0.11	16.93 ± 0.15	14.90 ± 0.13	15.17 ± 0.15	13.80 ± 0.13	16.60 ± 0.12
	Chlamydia trachomatis.	20.73 ±0.18	15.43 ± 0.14	12.70 ± 0.05	16.13 ± 0.11	13.70 ± 0.14	16.80 ± 0.12
100	*Escherichia coli*	20.72 ±0.03	11.73 ± 0.23	13.33 ± 0.15	14.23 ± 0.11a	15.73 ± 0.22	15.93 ± 0.16
	Acinetobacter baumanni	21.73 ±0.06	19.88 ± 0.17	19.70 ± 0.12	**22.80 ± 0.17***	12.83 ± 0.11b	**21.80 ± 0.12***
	Pseudomonas aeruginosa	21.45 ±0.06	18.46 ± 0.06	16.00 ± 0.11	16.27 ± 0.17	15.90 ± 0.21	16.83 ± 0.26
	Chlamydia trachomatis.	21.72 ±0.14	16.27 ± 0.13a	14.77 ± 0.06	15.30 ± 0.08	15.83 ± 0.07a	18.77 ± 0.23b
250	*Escherichia coli*	21.17 ±0.13	14.83 ± 0.14	15.67 ± 0.15b	17.40 ± 0.15b	17.77 ± 0.16	17.70 ± 0.21
	Acinetobacter baumanni	20.53 ±0.26	16.89 ± 0.11*	17.15 ± 0.06*	19.33 ± 0.13*	18.00 ± 0.11	20.03 ± 0.11b
	Pseudomonas aeruginosa	21.86 ±0.14	17.13 ± 0.06	16.10 ± 0.10	17.87 ± 0.07	16.03 ± 0.15a	19.03 ± 0.13
	Chlamydia trachomatis.	20.88 ±0.15	15.35 ± 0.06	13.11 ± 0.06	16.50 ± 0.06	18.03 ± 0.13	19.97 ± 0.12b
500	*Escherichia coli*	21.45 ±0.03	16.87 ± 0.77	18.18 ± 0.12a	19.80 ±0.12	19.87 ± 0.17	18.97 ± 0.16b
	Acinetobacter baumanni	22.73 ±0.12	14.83 ± 0.57	16.34 ± 0.06	18.50 ± 0.11	18.07 ± 0.16*	**21.30 ± 0.11b***
	Pseudomonas aeruginosa	21.16 ±0.17	18.10 ± 0.11a	18.13 ± 0.06*	20.23 ± 0.08	19.03 ± 0.26	**20.06 ± 0.09***
	Chlamydia trachomatis.	21.76 ±0.22	14.11 ± 0.09	15.16 ± 0.10	18.20 ± 0.13	19.13±0.16ab	19.07 ± 0.26

Values are Mean ± SD for three determinations

*Significantly ($p < 0.05$). Concentration of standard is 30 µg/mL of chloramphenicol

Table 3: Mean values of the zone of growth inhibition of flower extract of *Hibiscus moscheutosis* ofdifferent concentration on selected fungi (A).

Crude extract Diameter of the zone of inhibition in mm							
Concn (µg/mL)	**Control and Fungal (A)**	**Fluconazole**	**Hexane**	**DCM**	**Ethyl acetate**	**Chloroform**	**Methanol**
25	*Alternaria alternate*	24.12 ±0.13	**9.07 ± 0.00***	9.67 ± 0.17	10.19 ± 0.09	9.66 ± 0.13	10.19 ± 0.12
	Aspergillus glaucus	23.17 ±0.11	9.55 ± 0.23	9.89 ± 0.03	10.52± 0.07	9.47 ± 0.17*	10.45 ± 0.06
	Aspergillus niger	23.14 ±0.15	12.67 ±0.07	10.60 ± 0.04	11.87 ± 0.08	10.69 ± 0.15	11.33 ± 0.23
	Botritis cinerea	22.43 ±0.11	13.59 ±0.00	13.57 ± 0.12	12.0 ± 0.001	11.23 ± 0.17	10.16 ± 0.12
50	*Alternaria alternate*	23.17 ±0.13	16.68 ± 0.00	14.63 ± 0.18	13.35 ± 0.09	13.67 ± 0.09	12.11 ± 0.20
	Aspergillus glaucus	22.14 ±0.18	18.88 ± 0.00	17.10 ± 0.07	17.55 ± 0.11	16.99 ± 0.07	15.21 ± 0.00
	Aspergillus niger	23.15 ±0.18	15.97 ± 0.15	14.13 ± 0.11	17.13 ± 0.07	18.85 ± 0.11	16.70 ± 0.00
	Botritis cinerea	24.76 ±0.18	16.98 ± 0.06	16.88 ± 0.09	18.18 ± 0.09	19.57 ± 0.19	14.80 ± 0.10
100	*Alternaria alternate*	23.22 ±0.16	18.88 ± 0.06	14.67 ± 0.17	15.67 ± 0.15	16.68 ± 0.22	14.55 ± 0.06
	Aspergillus glaucus	22.88 ±0.09	20.79 ± 0.06	19.89 ± 0.06	**22.80 ± 0.13**	15.76 ± 0.08	**21.94 ± 0.21**
	Aspergillus niger	23.11 ±0.14	19.56 ± 0.06	18.78 ± 0.13	17.87 ± 0.08	19.89 ± 0.21	15.66 ± 0.06
	Botritis cinerea	22.16 ±0.14	20.67 ± 0.10	17.67 ± 0.12	18.70 ± 0.06	19.79 ± 0.05	15.85 ± 0.21
250	*Alternaria alternate*	22.77 ±0.23	18.77 ± 0.12	15.89 ± 0.08	19.10 ± 0.18	20.68 ± 0.15	16.89 ± 0.20
	Aspergillus glaucus	21.33 ±0.11	19.87 ± 0.06*	18.88 ± 0.09*	18.83 ± 0.13*	18.35 ± 0.14	21.03 ± 0.06b
	Aspergillus niger	23.16 ±0.11	16.48 ± 0.06	19.10 ± 0.16	17.11 ± 0.07	19.44 ± 0.13	19.03 ± 0.06
	Botritis cinerea	23.66 ±0.22	18.97 ± 0.06	20.17 ± 0.23	19.00 ± 0.03	19.43 ± 0.16	23.97 ± 0.06*
500	*Alternaria alternate*	22.76 ±0.16	20.18 ± 0.23	17.58 ± 0.15	20.12 ±0.12	19.96 ± 0.17	19.97 ± 0.06
	Aspergillus glaucus	23.47 ±0.13	16.15 ± 0.06	19.77 ± 0.19	18.37 ± 0.11	19.34 ± 0.08*	22.30 ± 0.10*

	Aspergillus niger	24.13 ±0.12	18.14 ± 0.10	20.23 ± 0.17*	17.34 ± 0.08	20.45 ± 0.05	23.14 ± 0.06
	Botritis cinerea	23.76 ±0.08	17.17 ± 0.06	19.00 ± 0.11	19.11 ± 0.15	18.22±0.07	19.11 ± 0.06

Values are Mean ± SD for six determinations

*Significantly ($p < 0.05$). Concentration of standard is 30 µg/mL of Fluconazole

Table 4: Mean values of the zone of growth inhibition of flower extract of *Hibiscus moscheutosis* ofdifferent concentration on selected fungi (B)

Crude extract Diameter of the zone of inhibition in mm							
Concn (µg/mL)	**Control and Fungal (B)**	**Fluconazole**	**Hexane**	**DCM**	**Ethyl acetate**	**Chloroform**	**Methanol**
25	*Alternaria alternate*	24.17 ±0.13	10.13 ± 0.06	8.55 ± 0.07	10.87 ± 0.06	9.67 ± 0.16	11.87 ± 0.13
	Aspergillus glaucus	22.27 ±0.12	9.13 ± 0.06	**8.47 ± 0.06**	10.57 ± 0.06	9.17 ± 0.17	11.57 ± 0.26
	Aspergillus niger	22.22 ±0.09	12.23 ±0.06	11.60 ± 0.00	11.67 ± 0.06	9.67 ± 0.09	12.73 ± 0.21
	Botritis cinerea	23.45 ±0.12	11.30 ±0.00	12.57 ± 0.06	13.0 ± 0.16	10.63 ± 0.08	14.63 ± 0.15
50	*Alternaria alternate*	23.56 ±0.14	10.40 ± 0.00	10.63 ± 0.15	12.87 ± 0.06	11.67 ± 0.07	13.00 ± 0.22
	Aspergillus glaucus	23.37 ±0.23	20.71 ± 0.00	17.50 ± 0.00	18.70 ± 0.10	12.73 ± 0.09	14.70 ± 0.04
	Aspergillus niger	24.44 ±0.34	13.43 ± 0.15	14.90 ± 0.10	14.77 ± 0.06	13.80 ± 0.11	15.60 ± 0.07
	Botritis cinerea	22.38 ±0.12	13.83 ± 0.06	11.70 ± 0.00	13.83 ± 0.06	12.70 ± 0.16	16.80 ± 0.13
100	*Alternaria alternate*	23.67 ±0.09	11.63 ± 0.06	10.67 ± 0.15	12.83 ± 0.12	14.73 ± 0.24	14.93 ± 0.17
	Aspergillus glaucus	23.56 ±0.07	19.33 ± 0.06	19.70 ± 0.10	**22.80 ± 0.10***	14.83 ± 0.05b	**23.80 ± 0.12***
	Aspergillus niger	24.67 ±0.13	16.27 ± 0.06	15.00 ± 0.10	15.87 ± 0.06	16.90 ± 0.21	18.83 ± 0.27
	Botritis cinerea	23.66 ±0.19	20.80 ± 0.10	12.77 ± 0.06	14.90 ± 0.00	12.83 ± 0.08	19.77 ± 0.23
250	*Alternaria alternate*	23.77 ±0.27	14.83 ± 0.12	15.93 ± 0.15	16.80 ± 0.17	13.77 ± 0.45	20.70 ± 0.26
	Aspergillus glaucus	22.87 ±0.45	16.73 ± 0.06*	17.93 ± 0.06*	18.73 ± 0.12*	14.00 ± 0.13	23.03 ± 0.09*
	Aspergillus niger	23.77 ±0.17	15.03 ± 0.06	16.10 ± 0.10	15.37 ± 0.06	15.03 ± 0.14	18.03 ± 0.09
	Botritis cinerea	25.59 ±0.09	13.87 ± 0.06	11.97 ± 0.06	14.24 ± 0.00	18.03 ± 0.16	19.97 ± 0.19
500	*Alternaria alternate*	23.68 ±0.05	14.87 ± 0.23	17.77 ± 0.12	18.15 ±0.10	19.87 ± 0.19	20.97 ± 0.13
	Aspergillus glaucus	23.74 ±0.07	15.83 ± 0.06	16.03 ± 0.06	19.20 ± 0.10	16.07 ± 0.09*	22.30 ± 0.17*
	Aspergillus niger	22.63 ±0.15	18.10 ± 0.10	18.23 ± 0.06*	18.23 ± 0.06	20.03 ± 0.17	19.06 ± 0.09
	Botritis cinerea	22.34 ±0.21	19.97 ± 0.06	14.00 ± 0.10	16.13 ± 0.10	21.13±0.07*	20.07 ± 0.11

Values are Mean ± SD for six determinations

*Significantly ($p < 0.05$). Concentration of standard is 30 µg/mL of Fluconazole

Table 5: Antioxidant compound and antioxidant activities of Hibiscus moscheutosis extracts.

Parameter	**Hibiscus moscheutosis**				
	Hexane	**DCM**	**Chloroform**	**Ethyl acetate**	**Methanol**
% DPPH inhibition	83.08±0.1a	96.67±0.77c	98.23±0.3d	89.78±0.6b	89.09±0.3a
FRAP values (µmoles Fe (II)/100g)	2447.03±184.3ab	2987.45±223.5c	2306.17±303.6b	2067.01±13.8a	2449.04±235.4ab
Total phenolics (mg GAE/100g)	4644.24±87.64	5522.34±166.89b	2574.35±449.4d	4766.23±188.2c	4886.16±118.3a
Total tannins (mg CE/100g)	2912.56±112.12c	4388.76±112.4d	1768.98±140.2b	2997.45±122.3c	2934.34±114.8b
Total flavonoids (mg CE/100g)	2088.67±131.2b	2833.16±179.6c	3748.88±159.4d	2234.56±176.4b	2223.12±124.3b
Total flavonols (mg QE/100g)	586.00±2.0c	340.77±3.3b	1288.67±49.2d	588.12±2.4b	641,02±1.2c
Total anthocyanins (mg c-3-QE/100g)	177.33±4.9c	213.55±2.9d	78.07±3.3b	166.7±6.6c	168.17±4.8c

Table 6: Colour analysis of Hibiscus *moscheutosis* flower extract.

Analysis	**Hibiscus moscheutosis**
L*	47.09 ± 0.03^{x}
A*	12.99 ± 0.05^{x}
B*	4.86 ± 0.04^{x}
C*	13.35 ± 0.08^{x}
Δh	19.89 ± 0.07^{x}

a* and b*: Chromatic components; [a]Lightness; [b]Chroma = $(a^{*2} + b^{*2})$ 1/2, Hue angle = arc tangent (b*/a*). All results expressed are mean of three individual replicates (n = 3±S.D) on dry weight basis Mean values followed by different letters in a row are significantly different ($p < 0.05$) from eachother.

Table 7: Evaluation of the FTIR spectrum of Hibiscus moscheutosis flower powder.

Frequency range (cm^{-1})	Hibiscus moscheutosis	
	Peak wavenumber(cm^{-1})	Functional group and origin
3380-3000	3352.99	O-H (Alcohol) stretch, H-bonded with intensity of strong bond, Polysaccharides
2950-2900	2973.98	C-H, (Alkane) stretch , strong intensity
2900-2845	2910.82	C-H, (Alkane) stretch , strong intensity
2800.2500	-	-
2500-1900	-	-
1700-1690	1736.15	C=O, (Ester) with strong stretch, lipid, triglycerides
1601-1600	1645.66	C=O, (Amide) stretch with strong intensity
1500-1483	1523.31	C=C, Carbonyl) with a stretch of strong intensity
1400-1300	1378.36	-C-H, (Alkane) with bending and a variable intensity
1100-1000	1083.91	C-O, of two bands or more strech
1000-998	1044.69	C-O, (Ether) stretch, with strong intensity
950-800	879.16	=C-H, (Alkene) with strong intensity

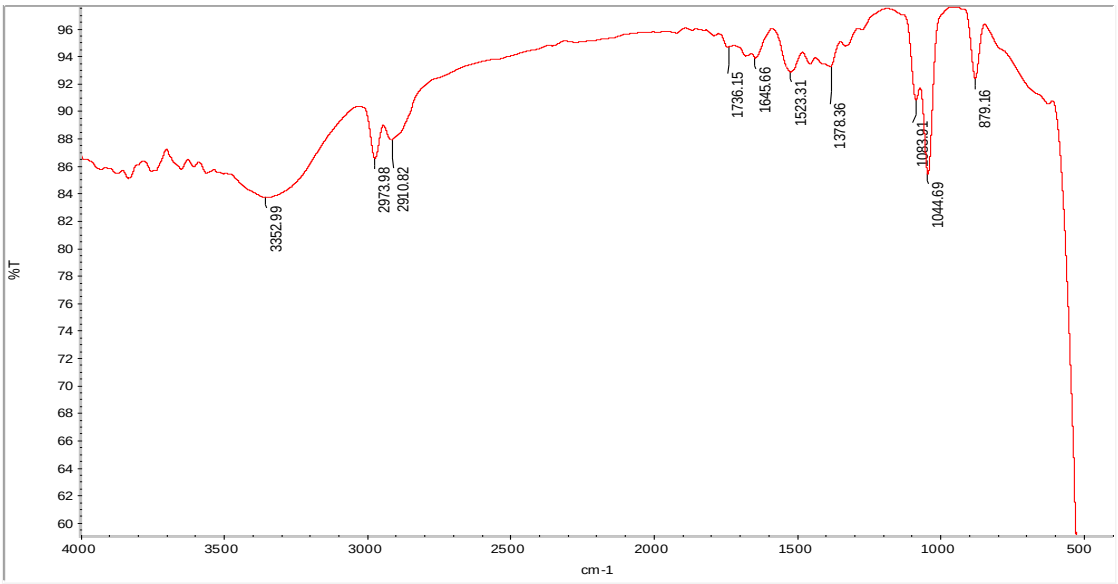

Figure 3: FTIR spectra of powdered flower petals of Hibiscus moscheutosis.

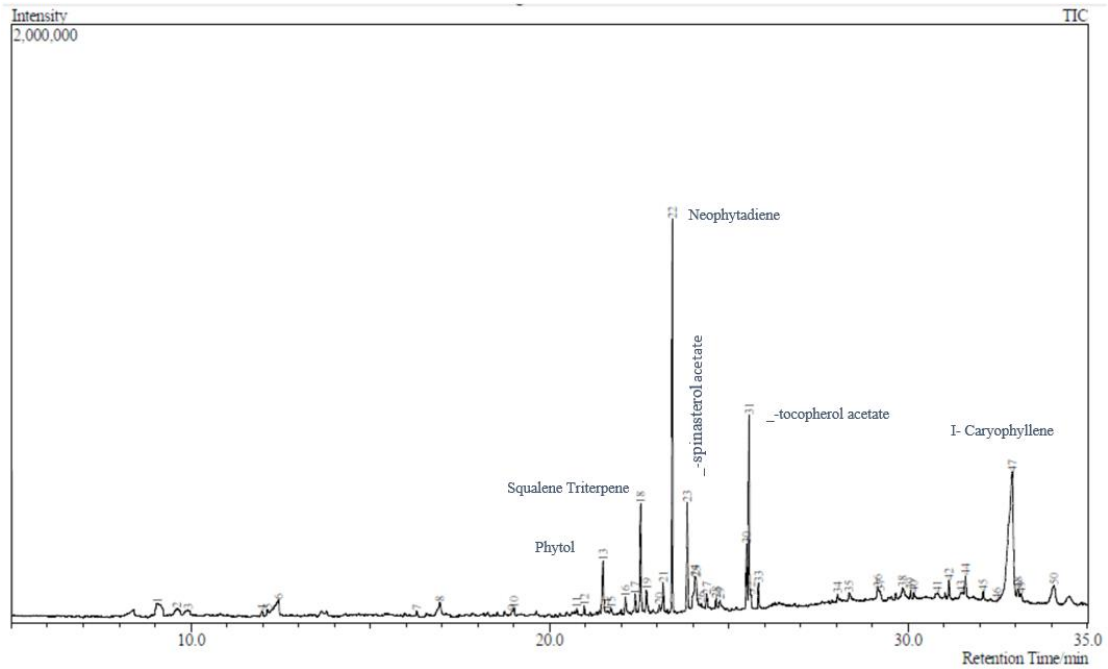

Figure 4: Ion chromatogram of Hibiscus moscheutosis methanol crude extract from GC-MS.

Table 8: Chemical composition in Methanol Crude of Hibiscus *moscheutosis.*

Peak#	R.Time	Area	Area%	Compound Name	Height%
1	9.072	453269	2.93	Cholesterol 3-O-[[2-acetoxy]ethyl]-	0.78
2	9.612	168135	1.09	1-Nonadecene	0.39
3	9.929	87850	0.57	Ethyl iso-allocholate	0.24
4	11.983	58811	0.38	1-Octadecene	0.32
5	12.140	46425	0.30	Pyrazolidine-3,5-dione	0.30
6	12.446	124982	0.81	1-Heptatriacotanol	0.81
7	16.295	44241	0.29	Loliolide	0.33
8	16.941	181987	1.17	Neophytadiene	0.70
9	18.950	47474	0.31	2-Hexadeoen-1-ol	0.29
10	19.014	51786	0.33	3,7,11,15-tetramethyl-2-hexadecen-1-ol	0.47
11	20.758	45105	0.29	phytone	0.41
12	20.966	57649	0.37	Hexadecanoic acid	0.59
13	21.489	492573	3.18	Phytol	3.41
14	21.600	43296	0.28	9,12-Octadecadienoic acid	0.18
15	21.709	67085	0.43	Linolenic acid	0.37
16	22.115	144924	0.94	ɤ-Elemene	1.11
17	22.388	106720	5.69	I- Caryophyllene	1.13
18	22.538	782287	5.05	Squalene Triterpene	7.09
19	22.702	221934	1.43	Heptacosane	1.44
20	23.059	51719	0.33	1,3,7-Nonatriene-1	0.47
21	23.168	280573	1.81	Geranyl linalool	1.97
22	23.424	2040765	13.17	Neophytadiene	25.72
23	23.842	830294	5.36	_-spinasterol acetate	7.09
24	24.061	476988	3.08	_α-Tocopherol	2.19
25	24.085	257920	1.66	Phytol	2.07
26	24.227	61591	0.40	10-Heneicosene	0.35
27	24.382	117763	0.76	Oxirane	0.88
28	24.644	59056	0.38	2-Pentadecanone	0.59
29	24.753	53946	0.35	Pentadecanal-	0.42
30	25.494	406535	2.62	Triacontane _-spinasterol acetate	4.25
31	25.560	1147427	7.41	_-tocopherol acetate	12.64
32	25.610	72892	0.47	Palmitaldehyde	0.92
33	25.823	162148	1.05	triacontane	1.64
34	28.033	46538	0.30	Squalene Triterpene	0.44
35	28.351	103216	0.67	Heptacosane	0.55
36	29.150	163428	1.05	Stigmasterol	0.78
37	29.215	68163	0.44	9-Octadecen-1-ol	0.52
38	29.843	162393	1.05	Chondrillasterol	0.63
39	30.063	51466	0.33	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.52
40	30.154	40563	0.26	n-Hexadecanoic acid	0.37
41	30.811	84135	0.54	Pentadecanoic acid	0.29
42	31.135	147253	0.95	Tetratriacontane	1.19
43	31.470	107562	0.69	2-Methyltetracosane	0.32
44	31.594	176078	1.14	Linoleic acid ethyl ester	1.37
45	32.086	59494	0.38	beta.-Sitosterol	0.50
46	32.475	42980	0.28	24-Norursa-3,12-diene	0.10
47	32.903	4301358	0.76	dl-.alpha.-Tocopherol	8.43
48	33.056	166933	1.08	Oleana-11,13(18)-diene	0.83
49	33.145	92813	0.60	Stigmasterol	0.48

3. DISCUSSION

Table 1 and 2 shows results obtained for the antibacterial activities of crude extract from five different solvent; hexane, dichloromethane, chloroform, ethyl acetate and methanol extract of Hibiscus *moscheutosis* flowers against Gram-positive and Gram-negative pathogenic bacteria. Itshows that at the concentration of 25, 50, 100, 250 and 500 mg/mL were observed to inhibit the growth of the pathogen (Bacillus subtilis, Listeria monocy togenes, Micrococcus luteus, Staphylococcus aureus) a Gram-negative bacteria at various values. Higher inhibition was observed with methanol crude extract on Listeria monocy togenes with inhibition value of 22.60 ±0.15 mg/mL and lower inhibition was observed with 25 mg/mL of 8.47 ± 0.14 mg/mL chloroform extract on Acinetobacter baumanni. Table 2 of Gram-positive bacteria (Escherichia coli, Acinetobacter baumanni, Pseudomonas aeruginosa, and Chlamydia trachomatis) shows lower inhibition on Acinetobacter baumanni bacteria with inhibition value of 8.33 ± 0.06 with DCM at 25 mg/mL higher inhibition was observed on

Acinetobacter baumanni of 22.80 ± 0.17 mg/mL.

The report available suggest the crude plant extract exhibited higher antibacterial activities with extract from the polar solvent than the non-polar solvents. This was attributed to the chemical constituent observed from GCMS profile of the methanol crude extracts. However, in the present study, flower extracts of Hibiscus *moscheutosis* inhibited both Gram-positive and Gram-negative pathogens equally. Polyphenols, flavonoids and tannins present in a sample might be responsible for the observed antibacterial activity. These compounds are generally produced by plants as a mode of defence against microbial infections.

It was reported that tannins were highlighted to be more effective against bacteria, yeast and fungi.[32] This was attributed to the complexes formed between tannins and microbial enzymes and cell envelope transport proteins. This complex eventually is believed to result in the inactivation of proteins resulting in inhibition of microbial growth.[33]

Table 3 and 4, the results indicate that the methanol crude extract of Hibiscus *moscheutosis* possessed the most potent antifungal activity, exhibiting a fungistatic effect on the growth of *Aspergillus glaucus* followed by ethyl acetate and chloroform, then hexane and DCM. As expected, higher concentrations of 250 and 500 mg/mL showed the highest inhibition value of 22.97 ± 0.06 mg/mL on Botritis cinerea at 500mg/mL while lower value was observed with 25 mg/mL chloroform crude extract on *Aspergillus niger* of 9.47± 0.17 mg/mL when compared to all the crude extracts and the control of 23.17± 0.11 mg/.mL. All crude extracts however, showed significant activities on all the fungiselected.

Table 5 shows the result obtained for antioxidant compounds and antioxidant assays expressed on a dry weight basis (d.w) With regard to visual colour, Table 6 the solvent extraction of Hexane, Dichloromethane, Chloroform, Ethyl acetate and Methanol of Hibiscus *moscheutosis* produced red colored extracts, respectively.

Thus, determining the antioxidant activity of Hibscus mosheutosis flower in this present study the extract of the polar and non-polar solvents exhibited rich scavenging effects on DPPH. The overall comparisons showed that the chloroform extract of 98.23±0.3 mg/mL to exhibit stronger scavenging effects on DPPH radicals, while hexane extract of 83.08±0.1 mg/mL had the lowest. Higher radical scavenging activity might be attributed to the presence of high phenolic, tannins or flavonols in the sample extracts.

The plant based phenol compounds was reported to have exhibit rich antioxidant activity by scavenging the free radicals generated during the normal metabolism process. This group encompasses a wide diversity of compounds, which mainly includes: flavonoids and proanthocyanidins.[34]

In the present study, the amount of total phenolics significantly varied between the solvents extracts (hexane, dichloromethane, chloroform, ethyl acetate and methanol) thus, total phenols ranged between 5522.34±166.89 to 2574.35±449.4 dmg GAE/100 g. from the Table 5 it was observed that Dichloromethane extract exhibited the highest value of 5522.34±166.89 mg.

The amount of Tannin in all the solvent extract differed significantly and ranged between 4388.76±112.4 to 1768.98±140.2 mg CE/100g. The tannin value was observed to be higher in Dichloromethane extract of 4388.76±112.4mg CE/100g and lower in chloroform extract of 1768.98±140.2mg CE/100g.

The presence of tannins in adequate amounts can be advantageous as they are able to quench free radicals very effectively, which in turn depended on the number of aromatic rings, molecular weight, and nature of the hydroxyl group substitution.[35] With regard to total flavonoid or bio-flavonoid content, chloroform extract showed high value 3748.88±159.4d mg CE/100 g and the least was found in hexane extract of 2088.67±131.2 mg CE/100 g. it was reported by Rice-Evan and Miller,[36] Flavonoids possess rich antioxidant properties and are produced as natural secondary metabolites in plants that encompass 6 sub-classes such as: isoflavones, flavonols, flavones and anthocyanins which vary in their structural characteristics. These flavonoids are capable of effectively interact and scavenge free radicals, which damage cell membranes and biological molecules.

The total flavonols, which are the most widespread sub-class of flavonoids in plant-based food-stuffs significantly varied between the solvent flowers extracts. High flavonol content was recorded in chloroform extracts of 1288.67±49.2 mg QE/100 g), while the lowest value was observed in Dichloromethane of 340.77±3.3 mg QE/100 g) this, indicating that chloroform to be more suitable for extracting flavonols compared to the other solvents.

With regard to total anthocyanin content, between the polar and non-polar solvent of the flower extracts Dichloromethane extract exhibited a higher value of anthocyanin 213.55±2.9 mg c-3-gE/100 g when compared to the other solvent extracts. It was in report that anthocyanins are becoming increasingly important not only due to their antioxidant properties, but also because of their antibacterial properties and use as a natural food colorant.[37]

Presence of high level of total phenols, flavonols, flavonoids, and anthocyanins has been reported in different flowers and their extracts.[37, 39, 40, 41] In this study it was observed that different solvent extraction systems can contribute significantly to differences in the

antioxidant activities of the extracts, this clearly illustrated that phenols including flavonoids, tannins, flavonol and anthocyanins are most probably the major contributor to the observed antioxidant properties in Hibiscus *moscheutosis* flowers.

In the colour analysis in this present study, lightness (L*) value of hibiscus was 47.09±0.03, Low L* value of hibiscus might be attributed to the dark colour of its flower petals, its chromatic component (a* and b*) values were 12.99±0.05 and 4.86±0.04, respectively. Chroma (C*) and hue angle (Dh) values are obtained from a* and b*. Chroma and hue values of hibiscus moscheutosis were 13.35±0.08 and 19.89±0.07 respectively. Chroma represents intensity of the colour, while hue angle values are stepped counter clockwise from red to purple (Dh =0) across a continuously fading colour circle through 90^{o} (yellow), 180^{o} (bluish-green) and 270^{o} (blue). Hibiscus showed lower chroma and hue angle values, which indicates that it has less intense coloured flower petals, this shows that the colour of Hibiscus *moscheutosis* flowers was dark (low L* value), but with low intensity (less vivid). Generally, in nature, a diversified group of flavonoids, anthocyanins, chlorophyll, xanthones, and betalains can contribute to the intense floral colour.[42] Differences in the floral colour depend entirely on the extent of co-occurrence with other colouring or pigment compounds and factors like chemical nature of pigments, their acylation and methylation status, pH of the vacuole, accumulation of the cyanidins or pelargonidin derivatives, and genetic inheritance.[43]

FTIR spectra and the functional group of compounds present in the powdered flower petals of Hibiscus moscheutosis shown in Fig. 3 and Table 7, respectively. The major functional groups included: polysaccharides, suberin, and lipid. Glycogen at 1378.36, and 879/15cm^{-1}, respectively. Also, FTIR spectra of the samples showed peaks in the range of 3352.99 cm^{-1} which could be the OH group of the phenolic compounds present in the samples.[44, 45] However, it is noteworthy to mention here that results obtained in FTIR alone are not sufficient to prove the existence of compound classes, especially when it comes to mixtures of many different compounds.

The phytochemical screening of methanol crude extract of GCMS analyses led to the identification of 49 chemical constituents in the methanol crude extract of *Hibiscus moscheutosis* flowers as shown in Figure 4 and Table 8. The identified compounds are arranged according to their elution order on silica capillary columns. The extract contains a complex mixture and the major compounds were dominated by squalene triterpene (13.17%), phytol (3.18%), Neophytadiene (5.36%), spinasterol acetate (7.41%), and tocopherol acetate (7.41%). The major compoundswere dominated by squalene triterpene (13.17%), Squalene is the most abundant in this crude extract. It is a naturally occurring triterpenoid and a precursor for the synthesis of secondary metabolites such as sterols, hormones, or vitamins. Squalene has been shown to have excellent antioxidant, anticancer, antibacterial, and antifungal biological activities. In pharmacognosy, squalene is extensively used as an excipient for disease management and therapy[46,47], found that squalene at high concentration resulted in disturbances in the fungus cellular membranes and interfered with essential membrane functions. The non-toxic chemical nature of lipids makes them excellent carriers as well as their ability to permeate the cell membrane of fungus due to their lipidic nature.

4. CONCLUSION

In conclusion, results of this study showed hibiscus flowers to encompass significant amount of antioxidant compounds, with the extracts exhibiting rich antioxidant activities. In addition, the flower extracts also possessed antifungal and antibacterial activity against various selected fungal, Gram-positive and Gram-negative bacterial pathogens. This study provides evidence that Hibiscus moscheutosis flowers extracts of solvents (hexane, Dichloromethane, Chloroform, Ethyl acetate and methanol) at 250 and 500 mg/mL had the highest antifungal activities as well as the selected Gram positive and Gram negative bacteria through in vitro and in vivo bioassays. The treatment altered the fungal and bacteria morphology and inhibited pathogens growth. The chemical constituents in this plant extract have the potential to be a natural antifungal and antibacterial agent. Results on antioxidants activity indicate the prospective of utilizing hibiscus flower extracts as a mode of natural agents for such pathogens and the results on colour analysis highlight the potential of utilizing these flowers as a natural colorant. With regard to functional group of compounds, the flowers showed the presence of polysaccharides, and lipids/ triglycerides. Thus, we propose an alternative disease management strategy using *Hibiscus moscheutosis* flower extract to control disease and infections either plant or human to cutile the menace of this pathogens. However, further research is necessary to elucidate the mechanism of action and develop the formulation to improve its efficacy and stabilityfor use in disease control.

Author Contributions: For research.

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Conflicts of Interest

The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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