



Phytochemical Screening and Antibacterial Activity of Five Malaysian Medicinal Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study, while author LA performed the laboratory experiments, statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JK and FA managed the analyses of the study. Author EY managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2014/12233

Editor(s):

(1) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.

Reviewers:

(1) Aline Augusti Boligon, Department of industrial pharmacy, Federal University of Santa Maria (UFSM), Brazil.

(2) Anonymous, University National of San Luis, Argentina.

Peer review History: <http://www.sciencedomain.org/review-history.php?iid=633&id=14&aid=5842>

Original Research Article

Received 24th June 2014
Accepted 18th July 2014
Published 22nd August 2014

ABSTRACT

Natural drugs play important and vital role in the modern medicine. It is usually used to cure some ailments which may not be treated by conventional medicine. Natural drugs may exhibit many biological activities, such as antimicrobial, anticancer, anti-diabetic and antioxidant. Five medicinal plants were screened, namely *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus*, for potential antibacterial activity against five clinical pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*). The purpose of this study is extracting, analyzing and screening phytochemicals and

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antibacterial activity in selected plant leaves. The ethanolic extracts of plant leaves were prepared using Soxhlet extraction and the *in-vitro* testing were conducted using disc diffusion method. The diameter of inhibition zones were measured in millimetre (mm), and test were conducted in three replicates. At concentration 5mg/mL, no inhibition zones detected in all extracts. As the concentration of extract increases, the bacterial inhibition zones also increases; thus, the more effective the antibacterial properties. The most active antibacterial plant was *P. ambonicus*, followed by *M. oleifera* and *C. citratus*; and the weakest were *C. dactylon* and *M. esculenta*. The most susceptible bacteria were *S. aureus*, followed by *K. pneumoniae* and the most resistant bacteria were *P. aeruginosa* and *Bacillus subtilis*. The phytochemical analysis revealed the presence of tannins, alkaloids, steroids, flavonoids and saponins in most of the plant extracts. The result of this study supports the use of all the selected five medicinal plants as a source of antibiotic substance for the possible treatment of human pathogenic organisms. These plants can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

Keywords: Natural drugs; *Moringa oleifera*; *Cymbopogon citrates*; *Cynodon dactylon*; *Manihot esculenta* and *Plectranthus ambonicus*; disc diffusion assay.

1. INTRODUCTION

Natural products play an important roles of drug discovery process include provide basic compounds affording less toxic and more effective drug molecules [1], serve as extremely useful natural drugs [2], exploration of biologically active prototypes towards newer and better synthetic drugs and modification of inactive natural products by suitable biological or chemical means into potent drugs [3,4].

Malaysia is one of the countries where vast areas of tropical rainforest are located. According to WWF (World Wide Fund for Nature) [5], forests in Malaysia still cover about 59.5% of the total land area even though deforestation has been increasing tremendously over the years [6]. This vast area of the forests has made Malaysia to become a country which has abundant natural resources and there are over six thousand species of tropical plants all over the country [7,8].

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs [9]. Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately [10]. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance [11]. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs [12,13].

Hence, the aim of this part of the study is to analyse the extract for the antibacterial property of *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus* [14,15].

2. MATERIALS AND METHODS

2.1 Materials

The herbal samples used in this study were; different parts of five chosen *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus*, collected from nursery at Klang, Malaysia. The tested bacteria obtained from (Dorset, England) and Merck (Darmstadt, Germany) Bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Antibiotic Disc Tetracycline, Gentamycin, Ampicillin. All other solvent and chemical used were of analytical grade from J. T. Baker (Phillipsburg, NJ, USA) [16].

2.2 Instrumentation

Soxhlet apparatus, first described in 1879, is a versatile tool that can be used to separate a single gram to hundreds of gram with 100% recovery [17]. The basic procedure calls for a solid sample to be placed in a porous container and allowing the condensed solvent to extract continuously. There are 3 basic components of a Soxhlet apparatus: a) condenser: to cool the solvent vapor and cause it to condense and turn back into liquid [18], b) porous container: to hold the liquid sample and allow for the condensed solvent to saturate and pass through thereby extracting active material [19], c) distilling pot: to hold the solvent ppo and serve as reservoir for the concentrated material [20]. Another instruments used were autoclave and biosafety cabinet from Ebsco[®], Rotary Evaporator, digital balance, and UV-visible Spectrophotometer [21].

2.3 Collection and Identification of Plants Materials

Fresh leaves of *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus* (See Fig. 1) were obtained from Jinjang Utara, Kuala Lumpur. Herbs Resources, Sungai Buloh, Selangor. All the plants were sent to Institut Biosains, Universiti Putra Malaysia for species identification.

2.4 Extract Preparation

The selected parts of the fresh plants were washed with running tap water and dried under sunlight for few weeks. All the dried plants were grinded by using blender to powdered-like substances [22]. The powdered-like substances were extracted with absolute ethanol for 24 hours using Soxhlet apparatus [23]. The extracts were then dried by using rotary evaporator until semi-solid is obtained. (See Fig. 2) [24].

2.5 Preliminary Phytochemical Screening

It involves the testing of the extracts of different Malaysian herbs to identify the various phytoconstituents [25]. The methods for the screening will be carried out by following the standard procedures described by Kokate et al. and Khandelwal KR [26,27] with some modifications. The tests for phytomchemical screening include:

2.5.1 Test for carbohydrates (molisch's test)

Extracts were dissolved individually in 5ml distilled water and filtered. Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube [28]. Add 0.2mL of concentrated

Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction [29].

2.5.2 Test for proteins & amino acids (millons test)

Test solution with 2mL of Millons reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) [30], white precipitate appears, which turns red upon gentle heating [31].

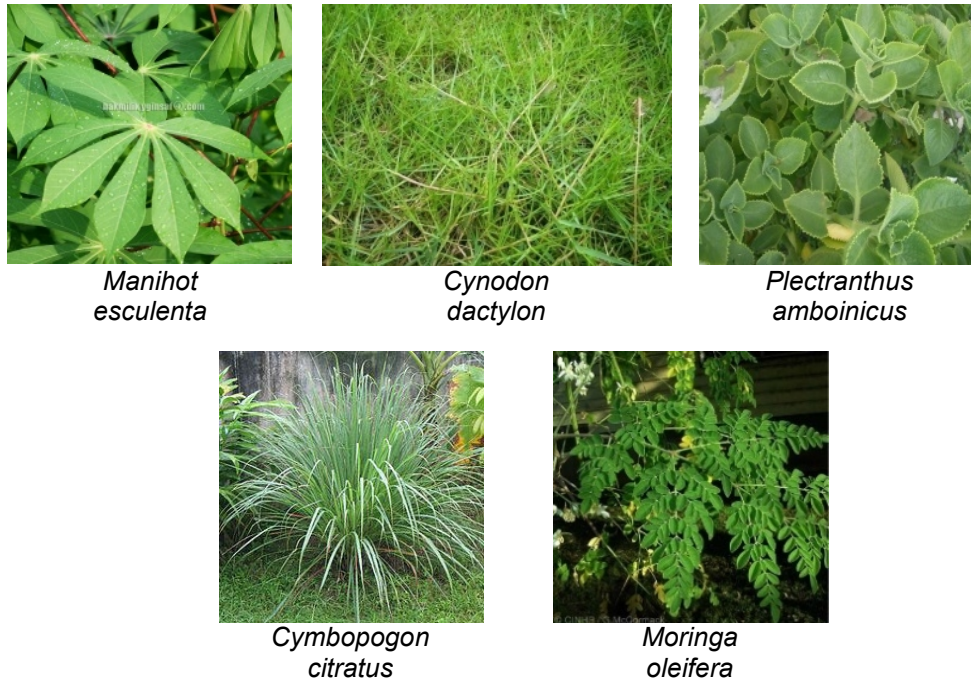


Fig. 1. The leaves of the selected five medicinal plants

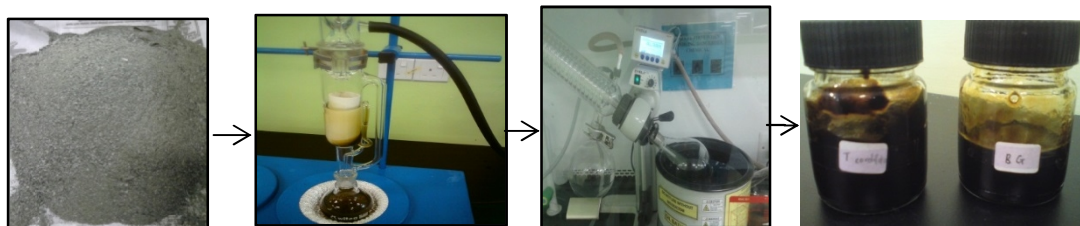


Fig. 2. Steps in extract preparation which includes grinding to form powder-like material, soxhlet extraction until rotary evaporation

2.5.3 Test for fats & fixed oils

- Stain test: Press the small quantity of extract between two filter papers, the stain on I filter paper indicates the presence of fixed oils [32].
- Saponification test: Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of Phenolphthalein separately

and heat on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats [33].

- c. Test for Alkaloids (Hager's test): Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were then treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids confirmed by the formation of yellow coloured precipitate [34].
- d. Test for Phytosterols (Salkowski test): Treat extract in Chloroform with few drops of cone. Sulfuric acid, shake well and allow standing for some time [35], red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of Triterpenoids [36].
- e) Diterpenes (Copper acetate test): Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [37].
- f) Test for Tannins and Phenolic compounds (Ferric Chloride Test): Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [38].
- g) Test for Flavonoids (Lead acetate test): Extracts were treated with few drops of lead acetate solution [15]. Formation of yellow colour precipitate indicates the presence of flavonoids [39].
- h) Test for Glycosides (Bromine water test): Extracts were treated with bromine water gives yellow precipitate [40].

2.5.4 Test for specific glycosides

- a) Saponin Glycosides-Frothing Test: Extract was dissolved in water and shaken vigorously [41]. Froth which last for a long time shows the presence of saponins.
- b) AnthraquinoneGlycosides–Borntragers Test: Extracts were treated with 5mL chloroform and shaken for 5 minutes [42]. The extracts were filtered and mixed with equal volume of 10% ammonia solution. A pink violet or red colour was observed for the presence of anthraquinone [43].

2.6 Disc Diffusion Methods

2.6.1 Media preparation

Three types of agar were prepared first, which is Nutrient agar, MacConkey agar and Mueller Hinton Agar for culturing the bacteria and testing its susceptibility [44]. Peptone water is prepared to be used in disc diffusion method [4].

2.6.2 Test organisms

The antimicrobial activity of the crude extract was screened against two gram-positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis* and three gram-negative bacteria; *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These organisms were collected from Microbiology Lab, MSU.

The bacteria were then sub-cultured into the newly prepared agar medium. *Staphylococcus aureus* and *Bacillus subtilis* were sub-cultured to Nutrient agar medium [45]. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were sub-cultured to MacConkey agar medium [46]. The petri dish were then incubated at 37°C for 24 hours. After incubation, the growth were examined and the petri dish were wrapped with parafilm and store in refrigerator under 2-8°C until further use [47].

2.6.3 Disc diffusion method

Mueller-Hinton agar medium is the only susceptibility test medium that has been validated by National Committee for Clinical Laboratory Standards (NCCLS) [8]. Mueller-Hinton agar should always be used for disc diffusion susceptibility testing, according to NCCLS and international guidelines [49].

3. RESULTS AND DISCUSSION

3.1 Plant Authentication

Collection of the selected plant materials was the first step in this study. Plant authentication was done in Institute Bioscience of University Putra Malaysia. From the result, it confirms that the collected leaves were from the plants of *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus* [50,8].

3.2 Extraction Yield

The selected plants were extracted with ethanol by using soxhlet extractor. The percentage of yield were calculated by using the formula [51]:

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract yield (g)}}{\text{Amount of dried plants used (g)}} \times 100$$

The results were summarized in the following (Table 1).

Table 1. Extraction yield of the selected plants

S. no.	Plant sample	Weight of dried plant (g)	Weight of crude extract (g)	Percentage yield (%)
1.	<i>Moringa oleifera</i>	37.01	10.35	27.97
2.	<i>Cymbopogon citrates</i>	51.71	9.27	17.93
3.	<i>Cynodon dactylon</i>	35.36	8.14	23.02
4.	<i>Manihot esculenta</i>	47.35	7.75	16.36
5.	<i>Plectranthus ambonicus</i>	50.74	8.95	17.63

3.3 Antibacterial Susceptibility Results

The antibacterial activity of ethanolic extracts was investigated using disc diffusion method, against five selected bacterial strains, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. All the examined extract showed varying degrees of antibacterial activities against the bacteria [52,53]. The bacterial inhibition zone were measured in millimeter (mm) using a ruler. Each test was done in three replicates, and the mean and standard deviation was then calculated. The diameter was measured edge to edge across the zone of inhibition over the centre of the disc [54]. The result of the five samples against five bacteria strains are shown in (Fig. 3). If no zone around the disc, it was reported as 0mm [55,56]. All the five plants *Moringa oleifera*, *Cymbopogon citrates*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus*

were tested to major reduction against five different bacterial strains [57]. The results of the screening of antibacterial activity is summarized in (Fig. 4).

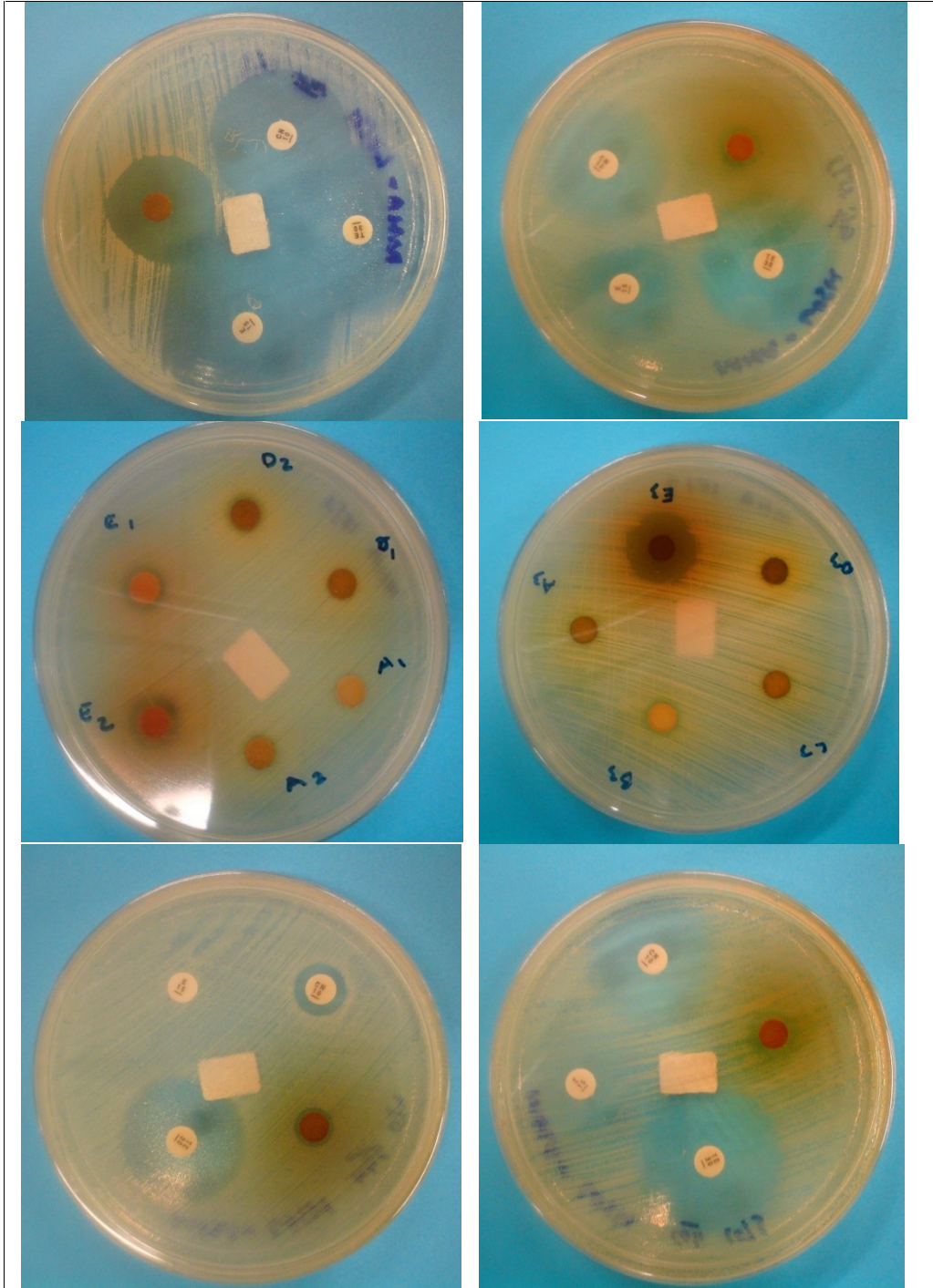


Fig. 3. Measurement of diameter of inhibition zone

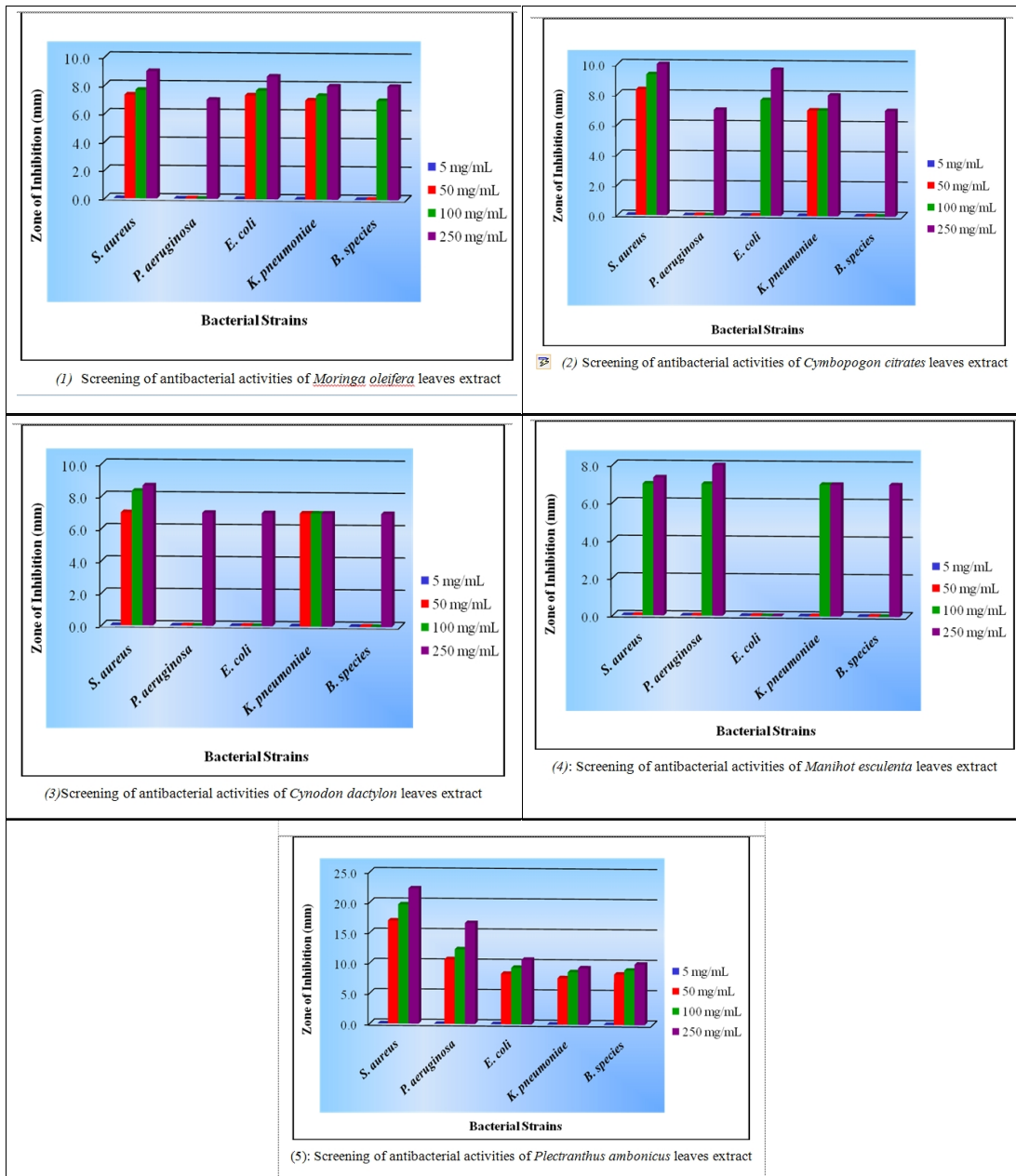


Fig. 4. The screening result of the whole five samples against five different type of bacterial strains

3.4 Phytochemical Constituents Analysis

Summary of the phytochemical analysis of ethanolic extracts of five different medicinal plants are shown in Table 2 below:

Table 2. Summary of the phytochemical analysis of ethanolic extracts of five different medicinal plants

S. no	Phyto-chemical	Test name	Observations				
			<i>M. oleifera</i>	<i>C. citratus</i>	<i>C. dactylon</i>	<i>M. esculenta</i>	<i>P. ambonicus</i>
1.	Carbohydrates	Molisch's test	+	+	-	-	+
2.	Protein & Amino Acids	Millons test	-	-	-	+	+
3.	Fats & Fixed Oils	Stain test	-	-	-	-	-
		Saponification test	-	-	-	-	-
4.	Alkaloids	Mayer's test	-	+	-	-	-
		Dragendroff's test	-	+	-	-	-
		Hager's test	+	+	+	-	+
5.	Steroids and Terpenoids	Salkowski test	+	+	+	-	+
6.	Diterpenes	Copper acetate test	-	+	+	+	+
7.	Tannins and Phenolic compounds	Ferric chloride test	+	+	+	+	+
8.	Phloba-tannins	Test with HCl	-	-	-	+	+
9.	Flavonoids	Lead Acetate Test	+	+	+	-	+
10.	Glycosides	Bromine water test	+	+	-	-	-
11.	Saponin Glycosides	Frothing test	-	+	-	+	+
12.	Anthra-quinone Glycosides	Borntragers test	+	+	+	+	+

Phytochemical constituents are secondary metabolites of plants that serve as a defence mechanism against many microorganisms, insects and other herbivores [58]. The preliminary phytochemical analysis revealed that all the plant extracts contain anthraquinone glycosides, and also tannins and phenolic compounds. These plants have one similarity which is they do not possess fats and fixed oils. Most of these plants contain alkaloids, tannins, steroids, flavonoids and saponins in the leaves, which could be responsible for the observed antibacterial property. These bioactive compounds are known to act by different mechanisms and exert antibacterial action in varying sensitivity [59,60].

4. CONCLUSION

This study has shown that leaf extracts of *Moringa oleifera*, *Cymbopogon citratus*, *Cynodon dactylon*, *Manihot esculenta* and *Plectranthus ambonicus* possess antibacterial properties against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Knowing the phytochemical constituents can help one to evaluate the medicinal values of the leaves. Flavonoids and tannins have antimicrobial and antioxidant properties. Alkaloids have pronounced physiological effects particularly on the nervous system. The presence of the main three phytochemicals in the leaves suggests that these plants are physiologically active, supporting the claim by traditional healers. These plant extracts could be promising natural antibiotics with potential applications in controlling bacteria that can cause diseases. Isolation, identification and purification of these phytochemicals and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating antibiotics should be the future direction for investigation.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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