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Phytochemical analysis and antimicrobial screening of selected Yemeni folk medicinal plants

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Abstract
For along period of time, medicinal plants have played a vital role in the treatment of many diseases. The present study was carried out to evaluate phytochemical analysis of aqueous, methanol, ethanol, and chloroform crude extracts and antimicrobial screening of four Yemeni folk medicinal plants. The phytochemical analysis of the plant extracts revealed presence alkaloids, tannins, glycosides, saponins, flavonoids, steroids, terpenoids, vitamin K and vitamin C in all study plants. The antimicrobial activity of solvent extracts of *Plectranthus asirensis, Plectranthus amboinicus Lavandula pubescens Decn*e, and *Dorstenia foetida* plants were inspected against the selected experimental pathogens such as *K. pneumoniae*, *E. coli*, *S. epidermis*, *S. aureus*, and C. *albicans* by agar well diffusion method. The results showed that extract from the investigated plants had antimicrobial activity in which ethanol and methanol extracts showed the highest values.

Keywords: Folk medicinal plants, phytochemical analysis, antimicrobials activity, leaves and rhizomes extracts

Introduction
The use of herbal remedies are widely used in about 80% of populations against many infection diseases, but only few of them have been studied chemically and biologically in order to identify their active constituents [1-3].
Nowadays, phytochemicals with adequate antimicrobial potential extracted from different parts of the plants can be used for the treatment of microbial infections such as diarrhea, dysentery, cough, cold, fever, bronchitis, cholera [4].
Identification of secondary metabolites from plants in therapeutic application of diseases is of growing interest as they contain many active phytochemical constituents. Bioprocess can convert simple compounds to complex compounds and it uses in several medicines and therapeutics [5].
Yemeni folk medicinal plants, *Plectranthus asirensis*, *Plectranthus ambinicus*, *Lavandula pubescens* Decne and *Dorstenia foetida* have been used for the treatment of several diseases such as rash and itching, malarial fever, renal and vesicle calculi, cough, chronic asthma, colic convulsions, epilepsy, psoriasis and vitiligo [6-12].
The contemporary research was done to evaluate phytochemical composition and antimicrobial potential of four medicinal plants.

Materials and Methods
Collection of plant materials
The selected plants were collected from their natural habitat in Yafae-Yemen. The plants were identified and authenticated by Prof. Abdul-Nasser Algfry in the Department of Botany, Education collage- University of Aden.

Chemicals
The entire chemicals used in the present study are of analytical grade.

Micro-organisms
Plant extracts were screened for their antimicrobial activity against four bacteria; two gram-positive bacteria (*Staphylococcus aureus* S.No.300-969-3374 and *Staphylococcus epidermidis* S.No.800-900-3376) and two gram-negative bacteria (*Escherichia coli* S.No.800-966-1793,

Klebsiella pneumoniae S.No.800-445-9890) and against one fungi (*Candida albicans*) obtained from the Department of Medical Microbiology-King Abdulaziz Hospital, Kingdom of Saudi Arabia.

Plant martials extraction

The plant parts (The leaves of *Plectranthus amboinicus, Plectranthus asirensis, Lavandula pubescens Decne* and the rhizomes of *Dorstenia Foetida*) were carefully washed under running tap water followed by sterilized water, cut into small parts and then air-dried under natural conditions for three weeks. The dried plant materials were powdered and sieved and each 50 g of plant extract (0.210-0.350 mm in size) were extracted with 500 mL of various organic solvents viz. water (AQ), methanol (ML), ethanol (EL) and chloroform (CF) for 48 hr at room temperature at orbital shaker through maceration method of extraction. The extracts were centrifuged at 2500 rpm for 15 min then filtrated through Whatman No.1 filter paper and concentrated to dryness under vacuum at 60 °C using rotary evaporator device under constant pressure. Only water extract was autoclaved at 121 °C then all obtained dried extracts were stored in refrigerator at 4 °C in labeled and airtight/sterile bottles until use.

Qualitative analysis

A preliminary phytochemical screening investigated the presence of alkaloids (Mayer's test and Dragendorff's test), glycosides (Keller-Killai test), flavonoids (Ethanol%95+ferric chloride 1% reagent, Shinadow's test, and KOH test), saponines (Foam formation), taninn (Ferric chloride 1% reagent), terpens (Salowski's test), steroids (Libermann test) and coumarins (NaOH 1% + UV light) according to standard methods [13-21].

Quantitative analysis Determination of alkaloids [22]

20 g of ethanol extract was dissolved in 5% HCl (50 ml). The mixture was centrifuged and the aqueous portion was transferred to a new tube and basified with NH4OH (pH 8-10). The aqueous (basic) portion was extracted with CHCl3 three times, and then concentrated under reduced pressure. Each sample was dried and weighed to determine the amount of alkaloid residues and expressed as a percentage of weight of sample analyzed.

Flavonoids determination [23]

10 g of each plant sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125 mm).

The filtrate was later transferred into a crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated.

Glycosides determination [24]

80 ml of 20% ethanol was added to 5g of grounded sample then the solution was shook for a 6 hr, and then filtered through Whatman filter paper 42. The extraction process was repeated for the deposit remaining after filtration in the same way to two more times. Leachate collection extracted from three operations strongly was shook with 25 ml of chloroform in a separating funnel. All abstracted chloroform collection of six operations was then evaporated in a rotary evaporator until the drought. Sludge remaining was melted in a mixture of chloroform and methanol (1: 1), then the solution was filtered and evaporated to dry on a hot water bath until the stability of the weight. Calculation was made to get glycosides amounts.

Saponins determination [25]

20 g of each grounded sample was put into a conical flask and 100 ml of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 hr. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride.

The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

Tannins determination [26]

0.5 g of plant powder was heated with 25 ml distilled water in a water bath for 30 min until boiling then centrifuged quickly at 2000 rpm for a period of 20 min. Transfer to a 100ml-volumetric flask. 20 ml of lead acetate solution (4%) was added and completed in size with distilled water up to the mark. The mixture was shaken for an hour, then filtrated through a filter paper (type Whatman No. 1). The filtrated was dried in a drying oven at 105 °C then ignited in furnace up to 500 °C for two hr. The residue was cooled and weighing process was repeated until reached constant of weight.

Volatile oils determination [27]

Dry plant powder subjected to hydro-distillation for 4 hr for extraction of oils. The oil samples were extracted three times with hexane (30 ml) and treated with anhydrous sodium sulfate (5-10 g). The supernatant was collected with a pipette and concentrated by rotary evaporator under reduced pressure to obtain the essential oil. Percentage quantity was then calculated.

Vitamin K determination [28]

0.125 g of accurately weighed dry plant powder was transferred into a10 mL- volumetric flask then 8 mL of CH3OH-CH2Cl2 (1:1, v/v) was added. After 15 min of ultrasonic extraction, CH3OH-CH2Cl2 (1:1, v/v) was added to the mark. The sample solutions was stored in the dark. Prior to injection, the solutions was filtered through a 0.2 µm membrane. 10 µl of this extract was injected in HPLC using Agilent 25 cm x 4.6 mm Zorbax SB-C8 column (column compartment: 30° C) and methanol-water (5:95) as a mobile phase. The flow rate was 1ml/ min.

Vitamin C determination by the method [29]

10 g of dry plant powder was mixed with a little distilled water and about 5 ml of 2 M sulfuric acid was added, subsequently introduced into ascorbic acid measurement device as much vitamin C by titration with iodine solution (0.05 M). The calculated result is compared with the standard ascorbic acid solutions.

Antimicrobial screening [30]

Based on the results of phytochemical analysis, the antimicrobial screening of the plant extracts was done using agar well diffusion method for aqueous, methanol, ethanol

and chloroform fractions.

The bacteria were seeded in Mueller Hinton agar while fungi was seeded in Sabouraud dextrose agar. The crude extracts (1 g in 5 ml of distilled water for water extracts or of DMSO solvent for organic extracts) were prepared in different concentrations (50, 100, 150, and 200 mg/ml.). The microorganisms were sub- cultured into prepared normal saline and incubated at 37° C for 30 min. The concentration of each suspension was obtained to form a turbidity that matched with scale 0.5 of Mac Farland's standard (1.5×108 cells/ml).

Wells were then bored into the sterile Petri plates of the seeded organism using sterile cork-borer of 6 mm in diameter. 100 µl of the extracts (Well per concentration) and allowed to stand for 1 hr at room temperature for proper diffusion. All plates were incubated at 37 °C and observed after 24 hr. The zones of inhibition were recorded in millimeters (mm).

Statistical analysis

Statistical analysis was performed by one- way analysis of Anova. Differences were accepted as statistically significant when $P<0.05$.

Results and Discussion

Phytochemical analysis of selected medicinal plants

The phytochemical analyses of different chemical constituents of selected plant species under study on qualitative basis were reported in Table 1. The four extracts (AQ, ML, EL and CF) showed many of the active groups in all study plants, whereas the two alcoholic extracts have contained most of the active components due to the high polarity. Results also showed qualitative statements in this aspect that the absence of chloroform extract alkaloids in all study plants is maybe due to polarity of the solvent.

The qualitative tests have shown that there are alkaloids, flavonoids, glycosides and saponines present in all study plants. Quantitative analysis on the other hand (Table 2 and Fig. 1), reveals the presences of alkaloids in all study plants was lower than the previous studies [33] while saponines in chloroform extract has been observed for *Plectranthus ambinicus* plant as same as Rashmi and his co-worker [34]. The results of phytochemical screening of *Plectranthus asirensis* plant were not harmoniously with what was recorded for the plant *P. glandulosus* and *P. rotundifolius Almentmyin* [35, 36] but, on the other hand, were consistent with what was found in *P. hadiensis* plant [37].

There was a well harmony in the qualitative phytochemical results of our work on *D. foetida* plant and that reported in [38] for *D. psilurus*. The absence of steroids in all study abstracts was noted and the qualitative detection of coumarin and flavonoids in plant *D. foetida* was a much clearer than the rest of the study plants. *Lavandula pubescens Decne* showed the existence of harmony with *Plectranthus asirensis* and *Plectranthus amboinicus*. This is may be due to the affiliation of these three plants to one family (i.e. *Lamaiceae*). The results of qualitative statements to *L. pubescens Decne* plant showed agreement with what is recorded in *L. officinalis* plant that belongs to the same genus [39].

Analysis of vitamin K contents in study plants by HPLC showed a small percentage of it (Fig. 2), excepts in *Dorstenia foetida* plant which appeared free from it. Also the studied plants in this research contained high level of vitamin C, particularly in *Plectranthus ambinicus* plant, make these plants important for antioxidants.

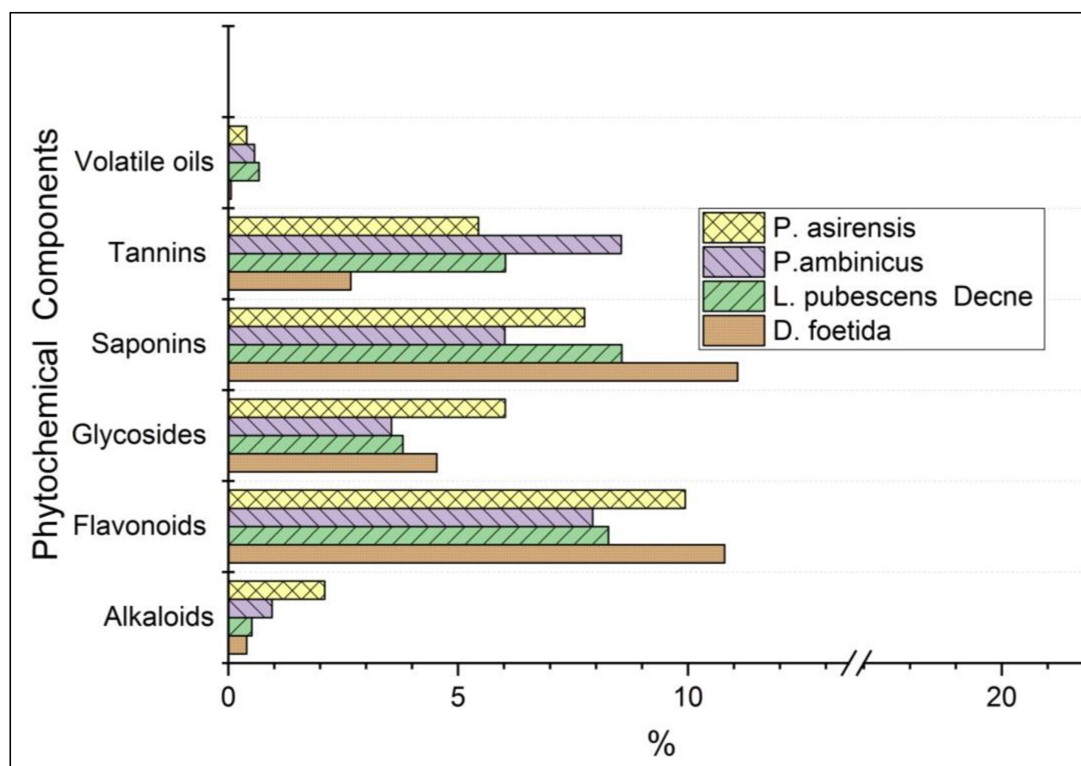

Fig 1: Quantitative phytochemical analysis in study plants

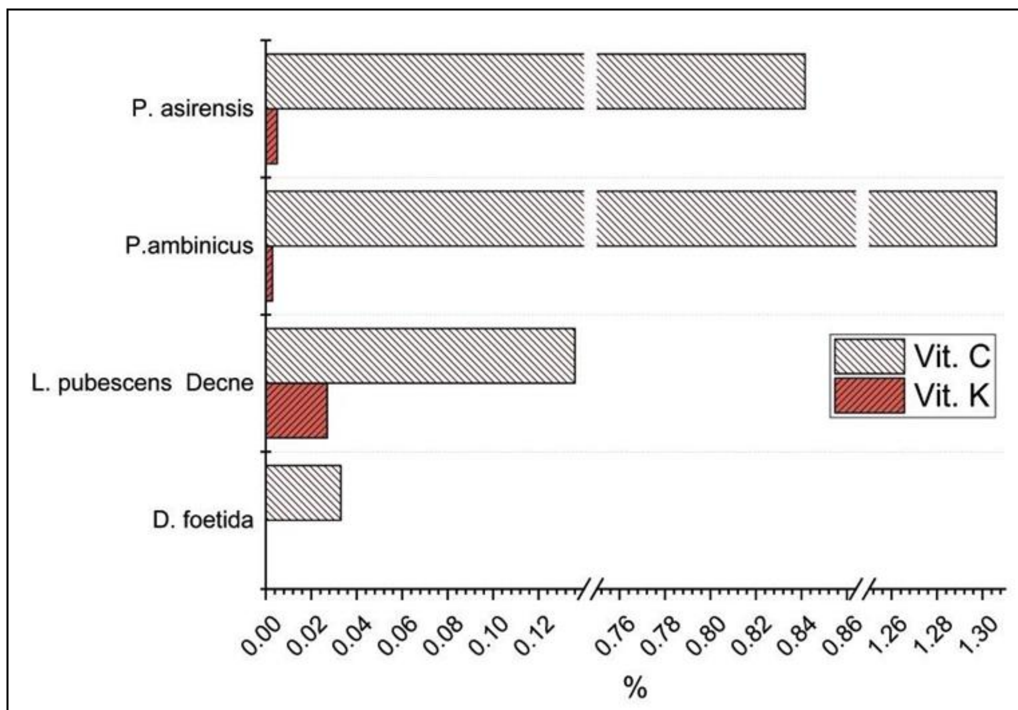

Fig 2: Vitamins C and K in the investigated plants

Antimicrobial screening

Tables (3-6) present antimicrobial screening of all plant extracts by the agar diffusion techniques. They have shown activity against testing microbes, and this effectiveness varied according to the type of extract (EL, ML, CF). Aqueous extract did not appear effective against tested microbes, except in *Plectranthus asirensiss* plant.

Both the ethanol and methanol extracts showed varying degree of antimicrobial activity against the test organisms. The ethanol extract for *Plectranthus asirensis* plant (Table 3) was found to be more effective than extracts against all the strains investigated.

The methanol extract for *Plectranthus amboinicus* plant (Table 4) was found to be more effective than others.

For *Lavandula Pubescens* Decne and *Dorstenia Foetida* plants the ethanol extract (Tables 5and 6) showed antimicrobial activity against all organisms tested.

Table 1: Phytochemical analysis plants study by reagents specific for the four extracts (ML: methanol, EL: ethanol, AQ: water, CF: chloroform)

Phytochemical compounds	Tests	Marks	*P. ambinicus*				*P. asiransiss*				*L. pubescens Decne*				*D. foetida*			
			ML	EL	AQ	CF	ML	EL	AQ	CF	ML	EL	AQ	CF	ML	EL	AQ	CF
Alkaloids	1- Mayer's test	Deep red precipitate	+*	+	+	-*	+	+	-	-	+	+	+	-	-	-	-	-
	2 - Dragendorff's test	Deep red precipitate	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
Glycosides	Keller Killai	Deep red	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	-
Flavonoids	1- Ethanol%95 + ferric chloride 1%	Bluish green color	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
	2 - Shinadow's Test	Pink color	+ +*	+	+ +	-	+ +	+	+ +	-	+	+	+ +	-	+ +	+	+	+
	3- KOH test	Yellow color	+ +	+	+ +	-	+ +	+	+ +	-	+	+	+ +	-	+ +	+	+	+
Saponines	Foam test	froth	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-
Taninn	Ferric chloride 1%	Blue-black	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Terpens	Salowski,s test	Reddish brown color	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
Steroids	Libermann test	Red color	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Coumarins	1% NaOH + UV light	Greenish yellow color	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*+ =Positive test, - =negative test, ++ = more positive test

Table 2: The quantitative phytochemical analysis in study plants

Plants	Alkaloids%	Flavonoids %	Glycosides %	Saponines %	Tanins %	Volatile oils %	Vitamin K %	Vitamin C %
Plectranthus asirensis	2.1±**(1)** 0.4a	9.94± 0.68a	6.027± 0.17a	7.75± 0.32a	5.44± 0.02a	0.4 ± 0.1a	0.157±0.005a	0.842±0.0058a
Plectranthus ambinicus	0.95± 0.04b	7.93± 0.97b	3.55± 0.30 b	6.02± 0.12b	8.55± 0.4 b	0.57± 0.058b	0.164±0.003 a	1.306±0.001b
Lavandula pubescens Decne	0.51± 0.03c	8.27± 0.26 b	3.80±0.22 b	8.56± 0.29c	6.03± 0.24c	0.67± 0.058c	0.4± 0.027 c	0.136±0.0058c
Dorstenia foetida	0.40± 0.025c **(2)**	10.8± 0.48a	4.54 ±0.2c	11.08± 0.19d	2.67± 0.25d	0.06±0.0058d	-	0.033±0.0053d
LSD	0.339	0.986	0.381	0.368	0.426	0.098	0.017	1.08

1. Values are means ± SEM (Standard error of means) of triplicate samples.
2. Same small letters (i.e. a, b, c, d) at the same column means there are no significant figures among the values.

Table 3: Concentration of *Plectranthus asirensis* extracts against the microbes

Test organism	EL extract mg/ml				ML extract mg/ml				AQ extract mg/ml				CF extract mg/ml			
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
K. pneumoniae	10.3± 0.58a	13.6± 0.58a	18.6± 0.58a	21± 1a	10.3 ± 0.58a	12.3± 0.58a	14.6± 0.58a	17.6± 0.58a	10.3± 0.58a	12.3± 0.58a	15.3± 0.58a	18.3± 0.58a	8± 1a	11.3± 0.58a	13.3 ± 0.58a	15.3± 0.58a
E. coli	11.6± 0.58b	14.6± 0.58b	18.3 ± 0.58a	20.3± 0.58b	9.6± 0.58b	11.3± 0.58b	13.6± 0.58b	16.6± 0.58b	12.6± 0.58b	14.6± 0.58b	16.6± 0.58b	18.6± 0.58a	10.3± 0.58b	12.3± 0.58b	14.3 ± 0.58b	17.3± 0.58b
S. epidermis	11.3± 0.58b	14.6± 0.58b	17.7± 0.57a	19.6± 0.57b	7.6± 0.58c	9.6± 0.58c	11.6± 0.58c	14.6± 0.58c	6± 1c	8.3± 0.58c	11.3± 0.58c	14.3± 0.58b	9.3± 0.58c	11.6± 0.58a	14.3 ± 0.58b	16.3± 0.58c
S. aureus	12.6± 0.58c	15.6 ± 0.58c	19. 3± 0.58b	23.6 ± 0.58c	11± 1d	12.6± 0.58d	15.3± 0.58d	18.6± 0.58d	9.3± 0.58d	12.6± 0.58a	14.6± 0.58d	16.3± 0.58c	10.6± 0.58b	13.3± 0.58c	15.3 ± 0.58c	17.3± 0.58d
C. albicans	-	-	8± 1c	11± 1d	-	-	6.6± 0.58e	9.6± 0.58e	-	-	6.3± 0.58e	9.6± 0.58d	-	-	7.3± 0.58d	9.6± 0.58e
LSD	0.764	0.764	1	1.146	0.662	0.764	0.854	0.854	0.936	0.764	0.854	0.854	0.936	0.764	0.85 4	0.854

1. Values are means ±SEM (Standard error of means) of triplicate samples.
2. Same small letters (i.e. a, b, c, d) at the same column means there are no significant figures among the values.

Table 4: Concentration of *Plectranthus ambinicus* extracts against the microbes

Test organism	EL extract mg/ml				ML extract mg/ml				AQ extract mg/ml				CF extract mg/ml			
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
K. pneumoniae	13± 1a	14± 1a	18± 1a	20.3± 1.53a	14.6± 0.58a	17.33 ± 0.58a	19.3± 0.58a	21.3± 0.58a	-	-	-	-	10± 0.58a	12.3± 0.58a	15±1a	16.6± 0.58a
E. coli	10± 1b	12.3 ± 0.58b	15± 1b	17± 1b	16.6± 0.58b	18.3± 0.58a	19.3± 0.58a	21.3± 0.58a	-	-	-	-	8± 1b	9.3± 0.58b	10.3± 0.58b	11.6± 0.58b
S. epidermis	5.67± 0.58c	8± 1c	10.3± 0.58c	12.6± 0.58c	9.6± 0.58c	10.6± 0.58b	13.7± 0.58b	15.3± 0.58b	-	-	-	-	9.3±0. 58c	10.6± 0.58c	11.6± 1.15b	12.6± 0.58c
S. aureus	7.67 ± 0.58d	9 + 1c	11± 1d	13.3± 0.58b	11.6± 0.58d	13.3± 0.58c	14.3± 0.58 b	16± 1b	-	-	-	-	9.3± 0.58c	10.6± 0.58c	13.3± 0.58c	14.6± 0.58d
C. albicans	5.3± 0.58c	8.6± 0.58c	10± 1 b	12.3± 0.58c	-	8± 1d	10.3± 0.58c	11.67± 0.58c	-	-	-	-	-	-	-	9.3± 0.58f
LSD	1.146	1.267	1.377	1.377	0.764	1.01	0.854	1.01	-	-	-	-	0.936	0.764	1.146	0.854

Table 5: Concentration of *Lavandula pubescens Decne* extracts against the microbes

Test organism	EL extract mg/ml				ML extract mg/ml					AQ extract mg/ml				CF extract mg/ml		
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
K. pneumoniae	11.6± 0.58a	13.3± 0.58a	15.6± 0.58a	18.3± 0.58a	10.3± 0.58a	12± 1a	14.6± 0.58a	16.3± 0.58a	-	-	-	-	-	7.6± 0.58a	8.6± 0.58a	10.6± 0.58a
E. coli	9.6± 0.58b	11.3± 0.58b	12.6± 0.58b	15.3± 0.58b	13.6± 0.58b	15.6± 0.58b	17.3± 0.58b	17.6± 0.58b	-	-	-	-	-	0	7.6± 0.58b	9.6± 0.58b
S. epidermis	9.3± 0.58b	11.6± 0.58b	13.3± 0.58c	14.3± 0.58c	10± 1a	12± 1a	14.6± 0.58a	16± 1a	-	-	-	-	-	7.3± 0.58a	9± 1a	10± 1 a
S. aureus	11.6± 0.58a	15.6± 0.58c	16.3± 0.58d	18.3± 0.58a	10± 1a	13.6± 0.58c	16.3± 0.58c	18.6± 0.58b	-	-	-	-	6.3± 0.58a	7± 0.58a	9.3± 0.58a	11.6± 0.58c
C. albicans	5.6± 0.58c	7.3± 0.58d	9.3± 0.58e	11.3±0. 58d	5.6± 0.58c	7.3± 0.58d	8.3± 0.58d	10.3± 0.58c	-	-	-	-	-	-	-	-
LSD	0.854	0.854	0.854	0.854	0.936	0.936	0.854	1	0	0	0	0	0.382	0.54	0.936	0.936

Table 6: Concentration of *Dorstenia foetida* extracts against the microbes

Test organism	EL extract mg/ml				ML extract mg/ml				AQ extract mg/ml				CF extract mg/ml			
	50	100	150	200	50	100	150	200	50	100	150	200	50	100	150	200
K. pneumoniae	8.3± 0.58a	10.6± 0.58a	13.3± 0.58a	15.6± 0.58a	9.3± 0.58a	10.6± 0.58a	12.3± 0.58a	14.6± 0.58 a	-	-	-	-	5.3± 0.58a	7.3± 0.58a	8.6± 0.58a	10.3± 0.58a
E. coli	6.6± 0.58b	7.3± 0.58b	9.6± 0.58b	12.6± 0.58b	4.3± 0.58b	6.3± 0.58b	8.6± 0.58 b	9.6± 0.58 b	-	-	-	-	-	-	-	-
S. epidermis	5.6± 0.58 c	8.6± 0.58c	11± 1c	12.6± 0.58b	4± 1b	5.3± 0.58c	8 ± 1b	9± 1b	-	-	-	-	-	-	-	7.6± 0.58b
S. aureus	7.3± 0.58d	10.3± 0.58 d	12.3± 0.58 d	14.6± 0.58c	4.3± 0.58b	6.6± 0.58b	9.6± 0.58c	11.6± 0.58 c	-	-	-	-	5.3± 0.58a	7 ± 1a	8.3± 0.58a	11± 1c
C. albicans	-	-	-	8.6± 0.58 d	-	-	-	8± 1d	-	-	-	-	-	-	-	-
LSD	0.764	0.764	0.936	0.854	0.662	0.764	0.936	1.146	0	0	0	0	0.54	0.54	0.54	0.662

References

1. WHO. Traditional medicinal Strategy, WHO, Geneva, 2002, 9.
2. Praveena Bhatt, Pradeep S Negi. Antioxidant and Antibacterial Activities in the Leaf Extracts of Indian Borage (*Plectranthus amboinicu*s), Food and Nutrition Sciences. 2012; 3:146-152.
3. Okusa PN, Penge O, Devleeschouwer M, Duez P. Direct and indirect antimicrobial effects and antioxidant activity. J. Ethnopharmacology. 2007; 112:476-48.
4. Joshi B, Sah GP, Basnet BB, Bhatt M, Sharma D, Subedi K *et al*. Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), *Achyranthes bidentata* (Datiwan) and Azadirachta indica (Neem). J. Microbiol. Antimicrob. 2011; 3(1):17.
5. Nirmala Babu Rao, Rajesh Goud Gajula DR, Sujatha E, Sita O. Phytochemical analysis and anti-microbial activity of costus igneus (insulin plant) leaf extract, IJBPAS, 2016; 5(6):1207-1214.
6. Lukhoba, Catherine W, Monique SJ, Simmondsb, Alan J Paton. Plectranthus: A review of ethnobotanical uses, Journal of Ethnopharmacology. 2006; 103:1-24.
7. Preeja G Pillai, Suresh P, Gitanjali Mishra M. Annapurna, Evaluation of the acute and sub acute toxicity of the methanolic leaf extract of *Plectranthus amboinicus* (Lour) Spreng in Balb C mice, European Journal of Experimental Biology. 2011; 1(3):236-245.
8. Al-Qurainy F, Khan S, Nadeem M, Tarroum M, Al-Ameri A. Selection of DNA barcoding loci and phylogenetic study of a medicinal and endemic plant, *Plectranthus asirensis* J.R.I. Wood from Saudi Arabia, Genetics and Molecular Research. 2014; 13(3):6184-6190.
9. Abdel-Mogib M, Albar HA, Batterjee SM. Chemistry of the Genus Plectranthus. Molecules. 2002; 7:271-301.
10. Abid Ali Ansari, Zahid Khorshid Abbas, Shalini Saggu, Hasibur Rehman, Mohamed M, Moawed. Growth responses of *Lavandula pubescens* to temperature regimes of Tabuk, Saudi Arabia, Journal of Medicinal Plants Studies. 2014; 2(6):38-41.
11. Fokunang CN, Mejane NN, Noumie E, Ngameni B, Gonsu HK, Kechia F *et al*. *In-vitro* evaluation of the anti-bacterial properties of crude extracts of *Dorstenia mannii* Moraceae, Journal of Microbiology and Antimicrobials. 2012; 4(6):88-95.
12. Bertin, Vouffo, Karsten Krohn, Simeon F Kouam, Hidayat Hussain Etienne Dongo, Kathrin Meier, Barbara Schulz *et al*. A new isobauerane-type triterpenoid and other, minor constituents from the twigs of Dorstenia dinklagei, Biochemical Systematics and Ecology. 2008; 36:655-658.
13. Sofowora EA, Olaniyi AA. Phytochemical examination of Dracaena manniistem bark. Planta Medica. 1975; 27:65-67.
14. Wagner H, Bladt S. Plant Drug Analysis. Springer, Berlin, 1996.
15. Harborne J. Phytochemical methods. A guide modern techniques of plant analysis. Chapman and Hall. London, 1973, 612.
16. Nasrabadi M, Halimi M, Nadaf M. Phytochemical Screeningand Chemical Composition of Extract of *Muscari neglectum,* Middle-East Journal of Scientific Research. 2013; 14(4):566-569.
17. Krishnaiah DT, Devi A. Bono and R. Sarbatly. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med Plants Res. 2009; 3:067-072.
18. Rahman Ur A, Malik S. Isolation and Structure Determination of Nigellaicin, Anavel Alkaloid from Seeds of Nigella sativ Tetrahedron. 1985; 26(23):27.
19. Sofowora EA. Recent trends in research into African medicinal plants. J. Ethnopharmacol. 1993; 38(2, 3):209-214.
20. Sofowora EA. Research on Medicinal Plants and Traditional Medicine in Africa. J Altern Complement Med. 1996; 2:365-372.
21. Aguzue, Onyinye Chinelo, Akanji, Fausat Temilola, Tafida, Muazu Abubakar *et al*. Comparative chemical constituents of some desert fruits Surajo in Northern Nigeria, Archives of Applied Science Research. 2012; 4(2):1061-1064.
22. Hina Fazal, Nisar Ahmad, Mir Ajab Khan. Physico-Chemical, Phytochemical Evaluation and DPPH-Scavenging Antioxidant Potential In Medicinal Plants Used For Herbal Formulation In Pakistan , Pak. J. Bot., 2011; 43:63-67.
23. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium. Pacific Sci. 1994; 48:458-463.
24. Farooqi, Azher Ali. Cultivation of medicinal and Aromatc groups, 2010, 166.
25. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 2001; 8b:203-208.
26. Dlaly Basil Kamel al-Hakim, Sadik Hassan. food analysis. Al Mosul University, 1987, 273.
27. Bhuiyan NI, Chowdhury SU, Begum J. Essential oil in roots of *Vetiveria zizanioides* (L.) Nash ex Small from Bangladesh. Bangladesh J. Bot., 2008; 37(2):213-215.
28. Dionex Corporation. Determination of Water- and Fat-Soluble Vitamins in Nutritional Supplements by HPLC with UV Detection. Application Note 251, LPN 2533, Sunnyvale, CA. 2010, 3.
29. British Pharmacopoeia, 2013, III. London.
30. Chrinius, Hammuel, Gary G Yebpella, Gideona, Shallangwa, ASABE M, Magomya *et al*. Phytochmical and Antimicrobial L Screening of Methanol and Aqueous Extracts of Agave Sisalana, Acta Poloniae Pharmaceutican Drug Research. 2011; 68(4):535-539.
31. Naga, Deepthi, Bolla, Praveen Kumar. Bhogavalli. Preliminary phytochemical screening and antibacterial studies of the flowers of *Antigonon leptopus*. Annals of Biological Research. 2010; 1(4):229-233.
32. Anesini C, Perez C. Screening of plant used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. 1993; 39(2):119-128.
33. Sreedharren B, Jaiganesh KP, Kannappan N, Sulochna N. Pharmacognostic studies on *Plectranthus amboinicus* Lour, Research J. Pharmaceutical, Biological and Chemical Sciences (RJPBCS). 2010; 1(4):413-424.
34. Rashmi, Sahay Khare, Shanta Banerjeeand Kanika Kundu. Coleus aromaticus benth-a nutritive medicinal plant of potential the rapeutic value. International Journal of Pharma and Bio Sciences. 2011; 2(3):488-500.
35. Egwaikhide PA, GimbAa CE. Analysis of the Phytochemical Content and Anti-microbial Activity of *Plectranthus glandulosis* Whole Plant, Middle-East Journal of Scientific Research. 2007; 2(3, 4):135-138.

36. Anbuselvi S, Hema Priya M. Physico chemical analysis of Plectranthus rotundifolius, Journal of Chemical and Pharmaceutical Research. 2013; 5(3):12-14.
37. Darsan B, Menon JM, Sasikumar. Pharmacognostic Study and Phytochemical investigation of Plectranthus Hadiensis. Int J Pharm Pharm Sci. 2011; 3(5):300-304.
38. VoukengIgor K, Victor Kuete, Jean P Dzoyem, Aimé G Fankam, Jaures AK Noumedem, Jules R Kuiateand Jean-Marie Pages. Antibacterial and antibiotic-potentiation activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes, BMC Research Notes. 2012; 5(1-10):299.
39. Asmma E, Al-Niaame, Raghad Akram Aziz. Study of *Lavandula officinalis* L. buds of flowers extracts activity against some species of multi-drug resistant clinical isolates of bacteria, Iraqi Journal of Biotechnology. 2013; 12(2):82-91.