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RESEARCH ARTICLE

PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF AERIAL PARTS AND ROOTS OF *PIMPINELLA CANDOLLEANA* WIGHT & ARNOTT

Kyi Kyi Oo[1,*], Ni Ni Zaw[3], Ei Ei Thant[2], Htay New Win[2], Thein Zaw Linn[2] and Soe Myint Aye[4]

[1]Department of Traditional Medicine, Ministry of Health and Sports, Myanmar
[2]University of Traditional Medicine, Ministry of Health and Sports, Myanmar
[3]Clinical Pathology Department, Mandalay General Hospital, Ministry of Health and Sports, Myanmar
[4]Department of Higher Education, Ministry of Education, Myanmar

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ABSTRACT

Infections still cause about one-third of all deaths worldwide and are the leading cause of death, mainly because of disease in developing countries. *Pimpinella candolleana* Wight & Arnott belonging to the family Apiaceae and *Shan kokkaya* in Myanmar was collected from Pindaya Township, Southern Shan State in 2015. The phytochemical, physico-chemical properties of crude aerial parts and root powdered were determined by Raaman (2006) and WHO (2011). The elemental compositions of both crude powders were analyzed by the Wavelength Dispersive X-ray Fluorescence. The antibacterial activity of the different concentrations of five extracts of the aerial parts and roots were assayed by agar disc diffusion method. In preliminary phytochemical investigation, flavonoids, glycosides, polyphenol, steroids, reducing sugar, amino acids and tannins were observed in root powder but reducing sugar, amino acids and carbohydrates are absent in aerial parts. In the physico-chemical analysis, the total ash content, acid insoluble and water soluble ash were presented and the both samples were more soluble in polar solvents. In elemental analysis, potassium, calcium, iron, phosphorus, chlorine, sulfur, magnesium, silicon, zinc and manganese were detected in both samples. The aqueous methanolic and pet-ether extracts of both samples had moderate activity on *Escherichia coli* and *Staphylococcus aureus* at 20 mg/disc concentration and pet-ether extract showed significant dose response relationship on *Staphylococcus aureus.* Therefore, this plant extracts possess various effective secondary metabolites, beneficial elements and antibacterial activity.

INTRODUCTION

Infections still cause about one-third of all deaths worldwide and are the leading cause of death, mainly because of disease in developing countries(Bryan 2011).In current years, multiple drugs resistance in human pathogenic microorganisms has residential due to haphazard use of saleable antimicrobial drugs usually used to the conduct of infectious disease. This position has required searching for novel antimicrobial substances from a variety of sources as novel antimicrobial chemotherapeutic agents and thus, has been rewarded newly to the biologically energetic compounds derived from herbal plants (Alwindy 2012).Traditional medicine based on plant materials are possible sources for new potent antibiotics for therapeutic use and an alternative treatment in infectious diseases. *P. candolleana* Wight & Arnott, family Apiaceae, is naturally grown in temperate and hilly region especially Shan State in Myanmar. These roots have been traded as a commercial product in Myanmar Crude Drug markets and local peoples use aerial parts as vegetables and roots as tonic. These dried roots are also composed of traditional medicine formulas which contributes to anti fever and anti-aching. Chang & Kang (2012) described that the most plants in *Pimpinella* genus have anticonvulsant, antispasmodic and relaxant effects, antioxidant, antibacterial and antifungul activities. In the present research, the antibacterial activity of different extracts of dried aerial parts and roots of *P. candolleana* Wight & Arnott were assayed on three pathogenic microorganisms; *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* which are common pathogens and resistant to antimicrobial agents. *E. coli* are the most common cause of urinary tract infection and diarrhea is extremely common worldwide. *P.aeruginosa* and *S.aureus* are common causes of human infections (Brooks *et al.* 2010).However, it is needed to prove antibacterial activity of the aerial parts and root extracts of this plant for the development of potentially useful antibacterial agents. Therefore, this study was undertaken to evaluate phytochemical properties and antibacterial activity of various extracts of the different parts of *P. candolleana* Wight &Arnott against on three selected pathogenic microorganisms.

***Corresponding author:* Kyi Kyi Oo,**
Department of Traditional Medicine, Ministry of Health and Sport, Myanmar.

MATERIALS AND METHODS

Sample Preparation: The plant specimens were collected from Pindaya Township, Sothern Shan state in Myanmar. It is situated between North latitude 20º 42' and 21º 13' and East Longitude 96º 25' and 96º 57' and the altitude is above 1100 m and identified according to Hooker (1879) and Menglan *et al.* (2005). The aerial parts and roots of *P. candolleana* Wight & Arnott were extracted with aqueous, ethanol, ethyl acetate, methanol and petroleum ether by using percolation method.

Phytochemical, Physico-chemical and Elemental Analysis of Aerial Parts and Roots: The preliminary phytochemicalandphysico-chemical properties of the crude powders of aerial parts and roots were carried out at Research Division, University of Traditional Medicine, Mandalay by the methods of Raaman (2006) and WHO (2011).The Wavelength Dispersive X-ray Fluorescence (WDXRF, Super Mini 200, Rigaku, Japan) was used to determine the elemental compositions of powdered aerial parts and roots sample at University of Research Centre (URC), Department of Geology, University of Mandalay.

Determination of Antibacterial Activity of Various Extracts
Antibacterial activity of the aerial parts and root extracts of *P. candolleana* Wight &Arnott was determined against three bacterial pathogens by the agar disc diffusion method (Kiehlbauch*et al.*, 2000) at the laboratory of Department of Microbiology at University of Medicine, Mandalay in 2015.

Test organisms: The test organisms; *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were supplied by Upper Myanmar Public Health Laboratory, Mandalay.

Preparation of culture medium and inoculums: Muller-Hinton agar was used as culture medium for antimicrobial susceptibility. The 70 ml of Mueller Hinton agar was poured into sterilized petridishes (15 cm dia.), shaken well and allowed to harden about 4 - 5 mm in thickness. The inoculums were prepared by making a direct broth (peptone-water) suspension of isolated colonies selected from an 18 to 24 hour agar plate with respect to direct colony suspension method. The turbidity of the resulting suspension was visually compared with 0.5 McFarland turbidity standards (1-2 x 10^8 CFU µg/ml).

Preparation of impregnated disc: The various crude extracts were diluted with distilled aqueous, 95% ethanol, methanol, ethyl acetate and pet-ether for different serial dilutions (100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml and 1000 mg/ml) using tween-80. The test extracts (20 µl) of each dilution were introduced into each disc of filter paper No.1 (6 mm in diameter) and dried at room temperature. Thus, the disc was completely saturated with the test extracts resulting 2 mg/disc, 4 mg/ disc, 6 mg/disc, 8 mg/disc, 10 mg/disc and 20 mg/disc respectively.

Antimicrobial testing: These prepared discs were placed on the surface of the inoculated agar plates. Standard disc of the antibiotic, cefotaxime (30 µg) was served as the positive antibacterial control. Negative controls were done by using paper disc loaded with 20 µl of respective solvents. These plates were incubated at 37ºC for 24 hours.

The antibacterial activity was evaluated by measuring diameter of the zone of growth inhibition in millimeters surrounding by discs. The experiments were done in duplicate sets.

Statistical Analysis: The mean values of the zone of growth inhibition were calculated. Pearson correlation method was used to observe the significance dose response relationship of the tested extracts on three control strains of bacteria and the *p* value was less than 0.05 was considered as statistically significant. Activity indices were calculated using the following formula to compare with control discs.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}}$$

RESULTS

Phytochemical and Physico-Chemical and Elemental Properties: The qualitative preliminary phytochemical constituents of crude powdered aerial parts and roots of *P. candolleana* Wight &Arnottare presented in Table 1. The physico-chemical properties values are presented in percentage as shown in Table 2. The macroelements and microelements involved in the powdered aerial parts and root are shown in Table 3.

Antibacterial Activities of the Various Extracts on Three Selected Bacteria: The antibacterial activities of aqueous, ethanol, ethyl acetate, methanol and petroleum ether extracts of aerial parts and roots of *P. candalleana* Wight &Arnott on *Escherichia coli*, *Pseudomonas aeruginosa*and*Staphylococcus aureus*were screenedat six different concentrations; 2 mg/disc, 4 mg/ disc, 6 mg/disc, 8 mg/disc, 10 mg/disc and 20 mg/disc respectively. The various extracts of both aerial parts and root has no activity on *E. coli* from 2 mg\disc to10 mg\disc but aqueous and ethanolic extracts of aerial parts exhibited mild antibacterial activity with 10 mm and 8mm and aqueous and methanolic extract of root showed sensitivity 11.5 mm and 8 mm respectively at 20 mg\disc concentration. The average zone of inhibition of standard (Cefotaxime 30µg\disc) showed 27.2 mm on *E. coli.*

There was no inhibitory zone in different concentrations of various solvent extracts of both aerial parts and root on *Pseudomonas aeruginosa* and even the average zone of inhibition in standard drug, cefotaxime was 15.4 mm. These results showed that there was no antibacterial activity at the different concentrations of both extracts of aerial parts and roots. The antibacterial activity of the different concentrations of various extracts of aerial parts and roots on *Staphylococcus aureus* were shown in the table 4 and figure 2. The 20 mg/ disc of methanolic extract of aerial parts showed 14 mm but aqueous and methanolic extract of roots with 8 mm in each inhibitory zone and the average zone of inhibition in standard drug, cefotaxime was 30.6 mm. The pet-ether extracts of aerial parts and roots showed significant dose related to antibacterial activity on *Staphylococcus aureus* by the method of Pearson correlation coefficient, r=0.913 ($p < 0.001$) and r = 0.957 ($p < 0.01$) with respect to each plant material. Therefore, it is indicated that the increasing dose of pet-ether extracts enhanced more significant antibacterial activity on *Staphylococcus aureus*. The inhibition zones of pet-ether extracts of aerial part and root were 14 mm and 12 mm at 20 mg/disc concentration.

Table 1 Preliminary Phytochemical constituents of crude powders ofaerial parts and roots

No.	Phytochemical constituents	Results	
		Aerial parts	Roots
1.	Alkaloids	−	−
2.	Flavonoids	+	+
3.	Glycosides	+	+
4.	Phenolic compounds	+	+
5.	Polyphenols	+	+
6.	Steroids	+	+
7.	Saponins	−	−
8.	Reducing sugar	−	+
9.	Amino acid	−	+
10.	Carbohydrates	−	+
11.	Acid / Base / Neutral	Base	Base
12	Tannins	+	+
13.	Cyanogenic substance	−	−

Table 2 Physico-chemical properties of crude powders of aerial parts and roots

No.	Physicochemical parameters	Quantity determined percentage	
		Aerial part	Root
1.	Moisture content	5.88%	6.54 %
2.	Total ash	3.75 %	8.23 %
3.	Acid insoluble ash	4.2 %	5.83 %
4.	Water soluble ash	88.54 %	93.86 %
5.	Water soluble matter	20.7%	33.4 %
6.	Ethanol soluble matter	12.8 %	18.8 %
7.	Methanol soluble matter	13.57 %	8.98 %
8.	Ethyl acetate soluble matter	6.65 %	3.36 %
9.	Pet-ether soluble matter	6.025 %	7.25 %

Table 3. Elemental compositions of crude powders of aerial parts and roots

Types	No.	Elements	Mass percentage	
			Aerial parts	Roots
Macroelements	1.	Potassium	33.90	30.40
	2.	Calcium	21.00	21.30
	3.	Iron	3.38	3.98
	4.	Phosphorus	1.41	1.77
	5.	Chlorine	2.59	1.44
	6.	Sulphur	0.76	1.40
	7.	Magnesium	0.86	0.588
Microelements	8.	Silicon	4.78	5.49
	9.	Aluminum	1.98	2.42
	10.	Manganese	0.515	0.853
	11.	Strontium	0.086	0.172
	12.	Zinc	−	0.139
	13.	Yittrium	−	0.0744
	14.	Titanium	0.523	−
	15.	Bromine	0.065	−

Table 4 Differences between antibacterial activity of aerial part and root extracts on *S. aureus*at different concentrations

Conc: mg\disc	Zone of inhibition (mm)									
	Aqueous		MeOH		EtOH		EtOAc		Pet-ether	
	AP	Rt	AP	Rt	AP	Rt	AP	Rt	AP	Rt
2 mg\disc	−	−	−	−	−	−	−	−	7	7
4 mg\disc	−	−	−	−	−	−	−	−	7	8
6 mg\disc	−	−	−	−	−	−	−	−	8	8.5
8 mg\disc	−	−	8	−	−	−	−	−	10	9
10 mg\disc	−	−	10	−	−	−	−	−	12	10
20 mg\disc	−	8	14	8	−	−	−	−	14	12
Solvent control	−	−	−	−	−	−	−	−	−	−
Standard (Cefotaxime 30µg\disc)	30	30	31	30	30	30	31	31	30	31

AP = Aerial PartsRt = Root

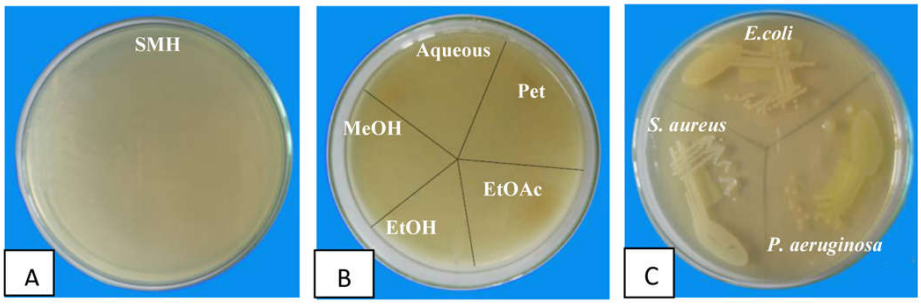

Figure 1 Preparation of Sterile Muller Hinton(SMH) agar plates, solvents plates and growth of colonies of three bacterial strains at 24 hours

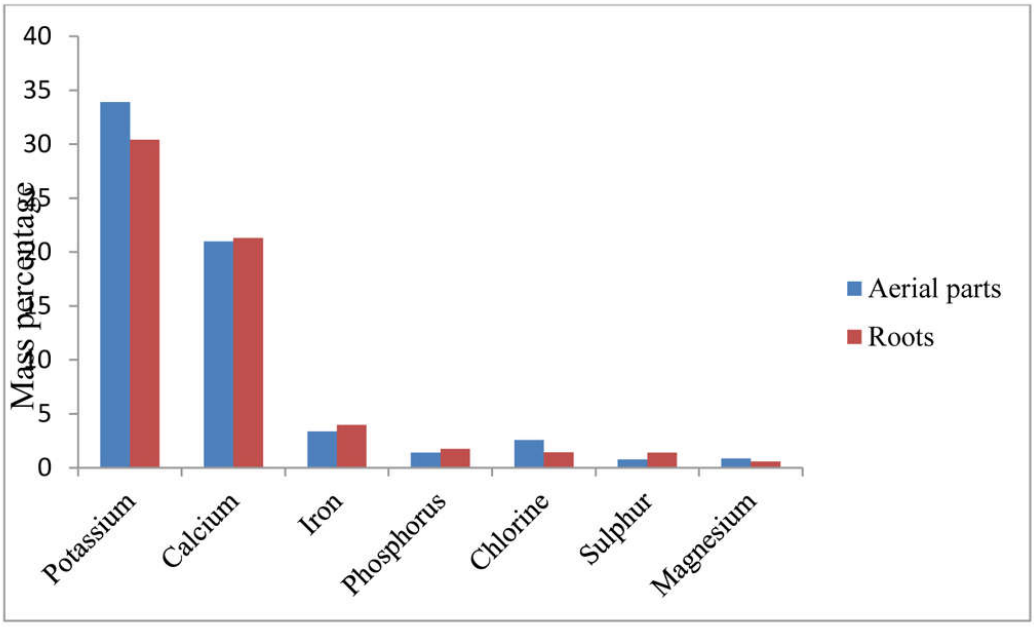

Figure 2 Macroelements of crude powders of aerial parts and roots

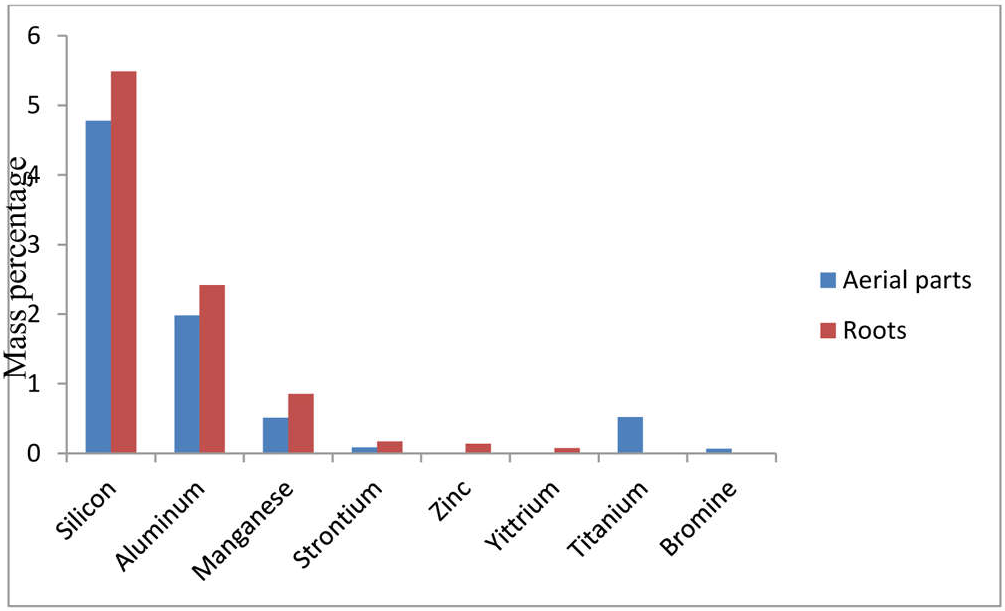

Figure 3 Microelements of crude powders of aerial parts and roots

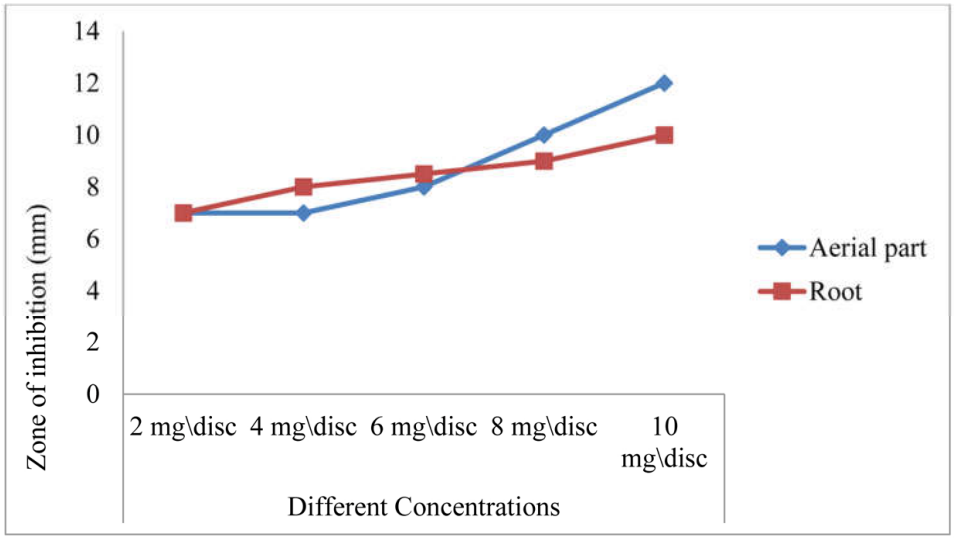

Figure 4 Dose response relationships in antibacterial activity of pet-ether extracts of aerial parts and roots on *S. aureus*

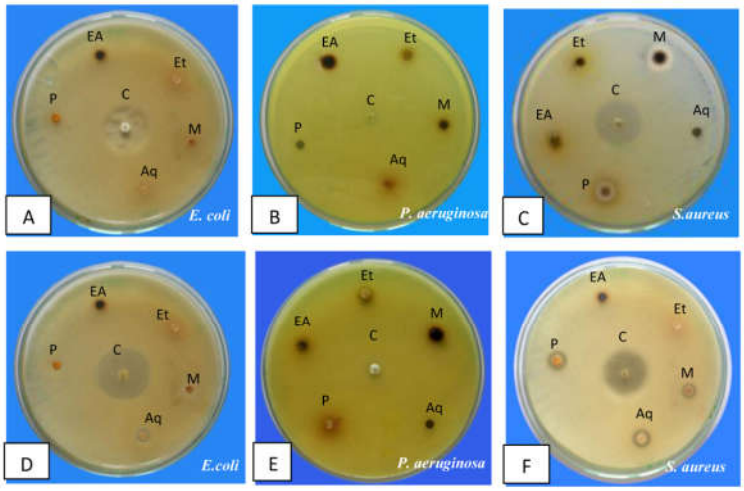

Figure 5 Antibacterial activities of various extracts of *P. candalleana* Wight & Arnott on three bacteria at 20 mg/disc concentration
A,B,C. Aerial part extract D, E,F. Root extract
W = Aqueous, M = MeOH, Et = EtOH, EA = EtOAc, P = Pet-ether, C = Cefotaxime

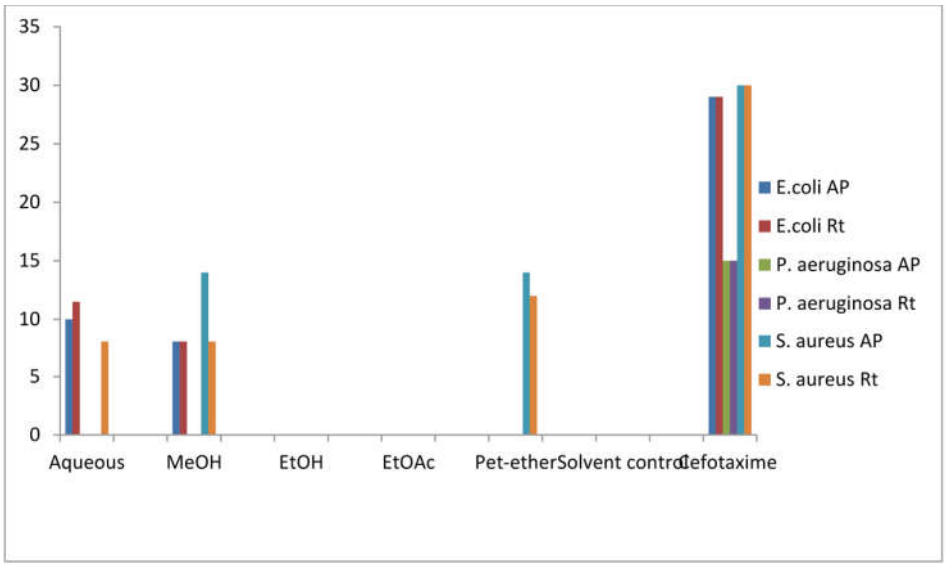

Figure 6 Comparison between antibacterial activity of aerial part and root extracts at 20 mg/disc concentration

At 20 mg/disc concentration, the aqueous and methanolic extracts of aerial parts showed zone of inhibition in activity index 0.35(10mm) and 0.28(8mm) on *Escherichia coli.* And the activity index of methanolic and pet-ether extracts on *Staphylococcus aureus*were 0.47. (14mm). In root extracts, aqueous and methanolic extracts showed in AI 0.4 (11.5mm) and 0.28 (8mm) on *Escherichia coli* and aqueous, methanolic and pet-ether extracts on *Staphylococcus aureus*in AI 0.27(8mm), 0.27 (8mm), 0.4(12mm) respectively. However, these results were lower antibacterial activity than standard cefotaxime.

DISCUSSION

The phytochemical methods are important to screen and analyze bioactive components not only for the quality control of crude drugs but also for the elucidation of their therapeutic mechanisms. The flavonoids, glycosides, polyphenol, steroids, reducing sugar, amino acids and tannins were observed in root powder but reducing sugar, amino acids and carbohydrates are absent in aerial parts. The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration of drugs. The total ash content, acid insoluble and water soluble ash were presented and the both samples were more soluble in polar solvents. This plant is rich in the macroelements; K, Ca, P, Cl, S, Mg, and microelements; Si, Al, Mn, Zn which are essential minerals in human metabolic processes. Kihira (2004) has noted that aluminum is a toxic element and cell loss can cause in the spinal cord and cerebrum of animals chronically given a low Ca, Mg and high Al diet. However, it was found that Al is very small amount and the content percentage of Ca and Mg was more than Al in this plant. The elements; Sr, Y and Ti were detected but its high concentration in the human body can cause side effect (Magili *et al.* 2014) and thus, this plant accumulate a little concentrations of these elements. Monitoring such medicinal plants for heavy metals concentration is of great importance in protecting the public from the adverse effects of these heavy metals. The elemental contents of plants are variable, due to the factors like differences between the plants species, localization and conditions of drying process.

The antimicrobial activities the various solvent extracts of aerial parts and roots of *P. candolleana* Wight & Arnott were tested on three control strains of bacteria such as *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus* by agar disc diffusion method. There had no activity in solvent control discs as negative control discs. It was observed that the standard drug, cefotaxime (30 µg/disc) was susceptible to *Escherichia coli* and *Staphylococcus aureus* whereas intermediate sensitive to *Pseudomonas aeruginosa* in accordance with the zone sizes denoted by CLSI (2012). The different concentrations of various extracts of both aerial parts and roots showed no activity against *Pseudomonas aeruginosa.* Although the antibacterial activities of other species of *Pimpinella* had been investigated, there was no literature about evaluation of the antibacterial activity about this species previously. This result is in agreement with Alwindy (2012) due to no activity on it in other *Pimpinella species.* The only 20 mg/discs concentration of aqueous and methanolic extract had mild antibacterial activity against *Staphylococcus aureus*. The all tested concentrations of pet-ether extract of both samples had moderate antibacterial activity on *Staphylococcus aureus* whereas no activity on *Escherichia coli.* Therefore, Gram positive bacteria which are more significant than Gram negative bacteria in their sensitivity to the oil of *Pimpinella* species described by Askari*et al.* (2009). The aqueous, methanolic and pet-ether extracts of both samples possess phytochemicals, macro and microelements which may be linked with the antibacterial activity exhibited. Tiwari *et al.* (2011) had presented the phytochemicals including phenolics, polyphenols, flavonoids and tannins have antimicrobial activity. These findings were pointed out that the pure compound showed higher antibacterial activity than crude extracts.

CONCLUSION

Traditional medicine based on plant materials are possible sources for new biologically energetic compounds derived from herbal plants as an alternative treatment in infectious diseases. It was observed that the plant *P.candolleana* Wight &Arnott possess phytochemical and physico chemical properties and nutritional elements which contribute to health benefits. It is also found that the various extracts of the different parts of this planthas antibacterial activity against on three selected pathogenic microorganisms. Therefore, these studies could be scientifically proved for the determination of phytochemical and physico chemical properties, elemental compositions and antibacterial activity of the aerial parts and roots of *P.candolleana* Wight &Arnott.

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GLOSSARY OF ABBREVIATIONS

AI - Activity Index
AP- Aerial parts
ATCC- American Type Culture Collection
CFU- Colony Forming Unit
Rt- Roots
SMH- Sterile Muller Hinton
WDXRF- Wavelength Dispersive X ray Fluorescence
WHO - World Health Organization

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