INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 2, p. 305-319, 2019

RESEARCH PAPER OPEN ACCESS

Nutraceutical potential and biological activities of selected medicinal plants

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Key words: Medicinal plants, proximate composition, Mineral profile, Phytochemical constituents, Antioxidant, Antimicrobial activity.

http://dx.doi.org/10.12692/ijb/15.2.305-319 Article published on August 24, 2019

Abstract

In this study five wild medicinal plants *viz. Caltha alba, Matricaria chamomilla, Ocimum basilicum, Phyllanthus emblica* and *Valeriana wallichii* were screened to examine their proximate composition, mineral profile, phytochemical constituents, antioxidant and antimicrobial activity. The results of proximate analysis showed that promising moisture (10.20%), fat (3.63%) and protein (11.50%) was recorded in *P. emblica* while ash (8.87%) and fiber (14.90%) was noted in *M. chamomilla* and *C. alba*. However, least amount of moisture (6.92%), fiber (6.10%) and protein (7.42%) was examined in *V. wallichii* whereas ash (5.07%) and fat (1.56%) was found in *C. alba* and *M. chamomilla*. Among all the plant species maximum NFE was observed for *V. wallichii* (70.19) followed by *M. chamomilla* (60.23%) while minimum value was reported in *P. emblica* (57.99%). The results of mineral profile showed that all the selected plants possess notable amount of C, O, Mg, Si, P, K and Al however, S and Cl was not detected in *V. wallichii* while Ca was not found in *O. basilicum*. Phytochemical screening revealed that among all the plant species promising total phenolics (95.25 mg GAE g^{-1}), total flavonoids (61.33 mg QE g^{-1}) and antioxidant activity (84.70%) was recorded in *P. emblica, V. wallichii* and *M. chamomilla* whereas lowest values was investigated in *O. basilicum* (10.25 mg GAE g^{-1}), *C. alba* (8.0 mg QE g^{-1}) and *V. wallichii* (65.40%). The antimicrobial potential of crude methanol extract of the examined plant species was tested against opportunistic bacterial and fungal strains by using well diffusion assay. Among all the plant species *M. chamomilla* exhibits promising inhibitory potential against *S. aureus* (74.07%) followed by *A. tumfaciens* (69.23%), *C. michiganensis* (59.25%) and *B. subtilis* (53.57%). Likewise, *V. wallichi* have considerable activity against *E. coli* (56.0%) and *X. compestris* (46.15%). In case of fungicidal activity notable inhibitory zone was recorded for *V. wallichii* and *P. emblica* against *Pacelomyces spp.,* (77.27% and 57.0%) followed by *V. wallichii* against *A. niger* (68.42%), however the selected plant also exhibited moderate to good and no activity against the tested fungal strains. It was concluded from the present results that antioxidant and antimicrobial potential of the plant extracts might be due to the presence of total phenols and total flavonoids content.

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Introduction

Plants are the fundamental module of selected recipes of folk medicines in remote areas of Pakistan. They are the key reserves for diets, medicines, pharmaceuticals intermediates, food additives and biological entities for various synthetic drugs [Abdul-Latif, 2011]. Medicinal plants and herbs are of great importance to the health of individual and communities. Herbal drugs are being used as remedies for various diseases across the world from ancient time [Abiy, Y, 2005, Abolaji, O.A, 2007, Adam, A.A, 2015]. In the recent past, increasing research evidence clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers [Agostoni, C, 1995]. Medicinal plants come into preparation of various drugs singly or in combination or even are used as the principal source of raw materials for the other medicines [Aiyelaagbe, O.O, 2009]. Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [Akpabio, U.D, 2013].

Each medicinal plant species has its own nutrients composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemical like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes [Akpabio, U.D, 2013, Akpanyung, E.O.1995]. The trace elements to be pharmacologically effective or essential, may need to be combining or chelated with some ligand, in order to be physiologically absorbed to prevent or cure impairment caused by deficiency of the element [Akpanyung, E.O.1995]. Active constituent of medicinal plants are metabolic products of plant cells and a number of trace elements play an important role in the metabolism [Ali, A. 2010]. Research has shown that some plants contain chemicals which are anti-nutritional and have the potential to help in reducing the risk of several deadly diseases in man [AOAC. 2012]. Reports show that these phytochemicals reduce LDL i.e. the cholesterol involved in depositing fat in the arteries, prevent blood clotting which can reduce the risk for heart attack or stroke. Sulfur compounds, which are examples of phytochemicals, are known also to reduce the cholesterol production in the body and through that keep the blood pressure down [Ayo, R.G. 2013].

Infectious diseases pose serious problems to health and they are main cause of morbidity and mortality worldwide. The uses of herbs in treatment of animal and human diseases have long been established. Most plant extracts have been shown to possess antimicrobial agents active against microorganisms in vitro. These plants contain medicinal properties which make them potent to cure or prevent diseases [Balandrin, M.F, 1985, Beaglehole, R, 2004]. Nowadays multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases [Bello, M.O, 2008]. This situation forced scientists to search for new antimicrobial substances. Presently, secondary plant metabolites have been extensively investigated as a source of medicinal agents [Bibi, S, 2006]. It is anticipated that phytochemicals with adequate antibacterial efficiency will be used for the treatment of bacterial infection. In the present research work five wild medicinal plants *viz. C. alba, Matricaria chamomilla, Ocimum basilicum, Phyllanthus emblica* and *Valeriana wallichii* were screened to determine their proximate analysis, mineral profile, phytochemical constituents, antioxidant potential and antimicrobial activity.

Materials and methods

Sampling of plant materials

In our study 5 wild medicinal plants *viz. C. alba, M. chamomilla, O. basilicum, P. emblica* and *V. wallichii* were collected from different areas of Abbotabad, Murree, Swat and Kashmir to analyze their nutraceutical potential. All the plants were identified

by eminent Taxonomist and were deposited at the Herbarium of Botany Department at the University of Peshawar. The fresh parts of the selected plants species were washed to remove debris, dust and other adhering materials. The plant samples were kept in shade for several days till complete dryness was achieved. Afterward the plant parts were crushed, grind and chopped with the help of electrical grinder. Clean plastic bags were properly labeled to pack the powder samples and stored at low temperature for further investigation.

Proximate analysis

Moisture: Moisture content in each plant samples was determined by a gravimetric method [Blackwell, W.H. 1990]. One gram of sample was pre-weighed (W1) in a beaker and placed in an oven at 105°C for 24 h. The sample was removed from the oven, cooled in a desiccator, and reweighed (W2). Moisture percentage was calculated according to the formula:

$$Moisture\ (\%) = \frac{W1 - W2}{W1} \times 100$$

Total ash: Total ash content was determined as total inorganic matter by incineration of a sample at 600°C [Blackwell, W.H. 1990]. Sample (1g) was weighed into a pre-weighed porcelain crucible and incinerated overnight in a muffle furnace at 600°C. The crucible was removed from the muffle furnace, cooled in desiccator and weighed. Ash content was calculated according to the following formula:

$$Ash\ (\%) = \frac{Ash\ weight}{Sample\ weight} \times 100$$

Crude fat: Crude fat was estimated by employing solvent extraction using a Soxhlet extraction unit [Blackwell, W.H. 1990]. One gram sample was weighed into an extraction thimble and covered with absorbent cotton. 50ml solvent (petroleum ether) was added to a pre-weighed cup. Both thimble and cup were attached to the extraction unit. The sample was subjected to extraction with solvent for 30 min followed by rinsing for 1.5 h. The solvent was evaporated from the cup to the condensing column. Extracted fat in the cup was placed in an oven at 110°C for 1 h and after cooling the crude fat was calculated using following formula:

$$Crude\ fat\ (\%) = \frac{Extracted\ fat}{Sample\ weight} \times 100$$

Crude fiber: Crude fiber in the samples was determined by the method described by [Blackwell, W.H. 1990, Cheeke, P.R. 1998]. Defatted sample (1g) was placed in a glass crucible and attached to the extraction unit. 150 ml boiling 1.25% sulfuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight.

The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original un-defatted sample and calculated according to the formula:

$$Crude\ fiber\ (\%) = \frac{Digested\ sample\ (W1) - Ashed\ sample\ (W2)}{Sample\ weight} \times 100$$

Crude protein: Crude protein was determined by the Kjeldahl method as described by [Blackwell, W.H. 1990]. Sample (700mg) was placed in a Kjeldahl digestion tube. Afterward 5g K_2SO_4 + 0.5g $CuSO_4$ and 25ml concentrated H_2SO_4 were added to the sample. The sample was digested for one hour. Now 20 ml deionized water was added to the sample after allowing it to cool. After adding 25ml NaOH (40%), the sample was then distilled and the ammonia liberated was collected in boric acid and titrated with 0.1N hydrochloric acid. A blank was prepared and treated in the same manner except that the tube was

free of sample. Protein percentage was calculated according to the formula:

$$\%N = \frac{(Sample\ titration - Plant\ titration) \times N \times 0.014 \times Dilution\ factor}{Weight\ of\ Sample \times V} \times 100$$

$$\%Crude\ protein = \%\ N \times 6.25$$

Elemental analysis

The elemental analysis was done at Centralized Resource Lab., University of Peshawar, using Energy Depressive X-Rays Spectroscopy (EDX), Model (Inca, 200), Company Oxford instruments made in U.K. The Sample was mounted rigidly on a specimen holder called a specimen stub. The stubs were used which were made of conductive materials such as aluminum, brass or copper. The stubs were coated with conductive tapes on both sides. The sample was mold on the conductor taps to stick. Then stub was put into the scanning electron microscope, which was connected to EDX and it was an analytical technique used for the elemental analysis or chemical characterization of a sample [Cheeke, P.R. 1998, Choudhary, R.K, 2011]. Identification of the elements is done by matching the peak values on the x-axis with known wavelengths for each element to reveal the sample's elemental composition.

Extraction of plant sample

The air-dried plants were exhaustively extracted with methanol using cold maceration techniques. About 50gm of powder samples were dissolved in approximately 100ml of methanol in separating funnel for several days and then filtered. The respective methanol extracts of each plant species was concentrated under reduced pressure at 40°C in the rotary evaporator. The crude methanol extract were kept in vials and stored in refrigerator at 4°C until analyzed.

Determination of total phenolic content

The total phenolic content in the crude methanol extract was determined according to a well-cited protocol [Chung, K.T, 1998]. A known amount of an extract was dissolved in 5mL of methanol from which 40µL was taken and dissolved in 3.16mL of distilled water. To this 200µL of Folin-Ciocalteu reagent was mixed and, after an interval of 8 min, 20% of 600µL sodium carbonate solution was added. The mixture was incubated at 40 °C for 30 min and absorbance was measured at 765nm on UV/Visible spectrophotometer. The standard calibration curve was prepared with Gallic acid standard solution (25 to 250mg L^{-1}) and the results were expressed as mg of Gallic Acid Equivalent (GAE) per g of dried mass of plant extract.

Determination of total flavonoid content

The total flavonoid contents in the samples were determined following the method reported by [Dastagir, G,2004]. The crude methanol extract was dissolved in 5 mL of methanol, from which 300µL were transferred into 3.4mL of 30% aqueous methanol. To this mixture, 150µL each of 0.5M $NaNO_2$ and 0.3 M $AlCl_3.6H_2O$ were added and mixed thoroughly. After the interval of 5 min, 1mL of 1 M NaOH was also added. The absorbance was observed immediately at 506nm against a blank. Quercetin was used as standard (25 to 250 mg/L) and the results were expressed as mg Quercetin Equivalent (QE) per g of dried mass of the extract.

Determination of antioxidant activity

Antioxidant activity was determined using the 2, 2-diphenyl-1-pycrihydrazyl (DPPH) radical scavenging assay reported by the standard procedure of [Davey, K.R. 1989]. In the procedure it is advised that a known amount of plant sample was extracted with known amount of methanol for 3 to 4 days. The excess solvent was removed by using rotary vacuum evaporator at 50°C under reduce pressure. Add 0.394 g DPPH in 1000 ml distilled water to prepared 0.001 molar solution of DPPH. Afterwards, 500 ppm stock solution of each plant sample was prepared. About 25 mg of each plant fraction was dissolved in 50 ml methanol. Mix 5 ml of stock solution with 25 ml methanol to make 100 ppm working solution. Afterward, add 3 ml of 0.001 molar DPPH solutions in one minute with 100 ppm solutions of sample and 1 ml of acetate buffer (pH 6.5). The solution was

vigorously shake and allowed to stand for 30 minutes. For control solution, add 3 ml DPPH with l ml acetate buffer solution. The % radical scavenging activity was determined using the following formula.

$$RSA\ (\%) = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100$$

Antibacterial activity

The antimicrobial activity of crude methanol extract was determined against oppurtunistic bacterial and fungal strains including *Agrobacterium tumefaciens, Bacillus subtilis, Clavibacter michiganensis, Escherichia coli, Staphylococcus aureus, Xanthomonas compestris Aspergillus flavus, Aspergillus niger, Trichoderma harzianum, Pacelomyces sp.* and *Fusarium oxysporum*.

Medium for bacterial culture

All the bacterial strains require nutrient agar media for their growth and culturing. About 2.8g of nutrient agar and 1.3g of nutrient broth were dissolved in distilled water and make the volume up to 100ml. About 7-8ml broths were decanted into test tubes and 20-25ml in conical flask. Afterward, sterilize the test tubes, flasks and media in autoclave at 121°C at 15 psi for 20 minutes.

Bioassay of antibacterial activity

The agar well diffusion method of [Desai, B.B, 1191] was used to evaluate the antibacterial activity. In this method the sterilized nutrient agar media was poured to the plates in a laminar flow unit after sterilization. With the help of sterile inoculation loop, streak the microbial stock culture on the plates. Now inoculate the microbial culture into broth and incubated in shaker for overnight. Microbial cultures (100µL) were transferred from the flask to test tube and the turbidity was adjusted according to MC Farland standard.

The standard microbial inoculums (50µL) were seeded on the prepared nutrient agar medium plates. Three wells were dug with the help of sterilized borer. The crude methanol extract (40µL) of each plant was applied to the respective wells.

The standard drug ciprofloxacin (1mg ml^{-1}) and DMSO was run parallel to the applied extracts. The petri plates were incubated at 37 °C for 24 hours. Finally the zone of inhibition of each plant fraction against the test microbial strains was measured. The percent zone of inhibition was determined by using the formula:

$$Inhibition\ (\%) = \frac{Inhibitory\ zone\ of\ extract}{Inhibitory\ zone\ of\ standard} \times 100$$

Medium for fungal culture

All fungal cultures were maintained on malt extract agar (MEA) medium plates. The MEA was prepared at concentration of 5% (w/v) in distilled water. It was then autoclaved at 121° C along with petri plates. The molten media was then poured to the plates in laminar flow unit.

Bioassay of antifungal activity

Antifungal susceptibility testing was done using the well diffusion method of [Dingman, S.L. 2002]. Fungal strains from the culture was inoculated to broth and then with the help of micropipette 100µl of inoculum was evenly distributed over the malt extract agar medium plates. The plates were allowed to dry for 15 minutes before use in the test. Wells were then created and a pipette was used to place 40µl of crude methanol extract of the said plant extracts into each well. The negative and positive controls were run in parallel with the plant extract. Pure DMSO was used as negative while Fluconazole and Itraconazole (1mg ml^{-1}) was used as positive control. The plates were incubated at 28 °C for 72 hours after which they were examined for inhibition zones.

Inhibition% = Inhibitory zone of extract\Inhibitory zone of Standard x 100

Results

Proximate composition

The results depicted in table 1 showed moisture, ash, fiber, fat, protein and NFE content of various plant species. It was observed that among all the plants highest moisture content was observed in *P. emblica* (10.20%) followed by *C. alba* (9.76%), *M. chamomilla*

(9.36%), *O. basilicum* (8.33%), and *V. wallichii* (6.92%). Likewise, promising ash content was found in *M. chamomilla* (8.87%) as compared to *O. basilicum* (8.27%), *P. emblica* (8.02%), *V. wallichii* (6.70%) and *C. alba* (5.07%). Highest crude fiber content was recorded in *C. alba* (14.90%) and *M. chamomilla* (12.51%) whereas lowest value was noted in *V. wallichii* (6.1%%). Highest fat content among the selected plant species was noted in *P. emblica* (3.63%) followed by *V. wallichii* (2.66%), *O. basilicum* (2.52%), *C. alba* (2.46%) and *M. chamomilla* (1.56%). In case of protein content maximum value was observed for *P. emblica* (11.50%) as compared to *O. basilicum* (9.82%), *C. alba* (8.90%), *M. chamomilla* (7.44%) and *V. wallichii* (7.42%). Likewise highest NFE value was recorded for *V. wallichii* (70.19%) followed by *M. chamomilla* (60.23%) while lowest value was noted for *P. emblica* (57.99%) respectively.

Table 1. Proximate composition of selected wild medicinal plants.

Plant species	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)	Protein (%)	NFE (%)
C. alba	9.76	5.07	14.90	2.46	8.90	58.89
M. chamomilla	9.36	8.87	12.51	1.56	7.44	60.23
O. basilicum	8.33	8.27	11.60	2.52	9.82	59.44
P. emblica	10.2	8.02	8.65	3.63	11.5	57.99
V. wallichii	6.92	6.70	6.1	2.66	7.42	70.19

Minerals analysis

The results depicted in table 2 divulged that among all the plant species maximum carbon, oxygen and potassium was noted in *O. basilicum* (59.99%), *P. emblica* (50.38%) and *C. alba* (1.91%) while lowest amount was recorded in *O. basilicum* (O = 33.96%; K= 0.40) and *P. emblica* (C = 46.92%). Highest value of magnesium (0.31%), aluminum (0.89%), silicon (2.16%) and phosphorus (0.71%) was examined in *V. wallichii* whereas lowest values was observed in *P. emblica* (Mg = 0.10%), *O. basilicum* (Al = 0.09%, Si = 0.13%) and *M. chamomilla* (P = 0.12%). Similarly promising amount of sulfur was recorded in *P. emblica* (0.20%) followed by *M. chamomilla* (0.19%) while no value was detected in *V. wallichii*. Maximum calcium (0.58%) and chlorine (0.54%) was found in *M. chamomilla* however no value was detected in *O. basilicum* and *V. wallichii*. It was concluded from the results that all the selected plant species possess considerable amount of minerals which are primarily responsible for various physiological functions of the body.

Table 2. Minerals profile of selected wild medicinal plants.

Plants species	Elements									
	C	O	Mg	Al	Si	P	S	K	Ca	Cl
C. alba	51.95	44.93	0.29	0.23	0.29	0.22	0.10	1.91	0.43	0.34
M. chamomilla	48.81	45.72	0.30	0.44	1.27	0.12	0.19	1.69	0.58	0.54
O. basilicum	59.99	33.96	0.27	0.09	0.13	0.41	0.15	0.40	NT	0.09
P. emblica	46.92	50.38	0.10	0.13	0.19	0.15	0.20	0.83	0.42	0.15
V. wallichii	49.3	46.01	0.31	0.89	2.16	0.71	NT	0.55	0.49	NT

Phytochemical analysis

The results depicted in Table 3 divulged that among all the plant species highest phenolic content was recorded in *P. emblica* (52.75 mg GAE g^{-1}) followed by *V. wallichii* (52.75 mg GAE g^{-1}) whereas lowest value was observed in *O. basilicum* (10.25 mg GAE g^{-1}). In case of flavonoid moiety promising amount was found in *V. wallichii* (61.33 mg QE g^{-1}) as compared to *P. emblica* (34.67 mg QE g^{-1}) while lowest concentration was observed in *C. alba* (8.0 mg QE g^{-1}) respectively. Likewise, promising antioxidant activity was reported in *M. chamomilla* (84.70%) followed by *P. emblica* (81.4%) whereas lowest value was recorded in *V. wallichii* (57.1%).

The results depicted in Table 4 showed that among all the plant species highest inhibitory zone of methanol extracts against *A. tumefaciens* was noted for *M. chamomilla* (69.23%) followed by *V. wallichii* (54.38%), *C. alba* (42.30%) whereas *O. basilicum* and *P. emblica* exhibited no activity against the test strains. The promising activity against *B. subtilis* was recorded for *M. chamomilla* (53.57%) followed by *C. alba* (50.0%), *O. basilicum* (32.14%), however *P. emblica and V. wallichii* failed to showed inhibitory zone.

Table 3. Total phenolics, total flavonoids content and antioxidant activity of crude methanol extract of different plant species.

Plant species	Total Phenolic Content (mg GAE g^{-1})	Total Flavonoids Content (mg QE g^{-1})	Antioxidant activity (%)
C. alba	15.25	8.0	68.80
M. chamomilla	40.25	31.33	84.70
O. basilicum	10.25	11.33	68.50
P. emblica	95.25	34.67	81.40
V. wallichii	52.75	61.33	65.40

In case of *Clavibater spp.,* considerable bactericidal activity was examined for *M. chamomilla* (64.81%) whereas other the test strains was resistible to methanol extract of other plants. Similarly, maximum zone of inhibition against *E. coli* was observed for *V. wallichii* (56.0%) as compared to *O. basilicum* (54.0%) and *P. emblica* (48.0%) while the test bacterial strain showed strong resistibility toward *C. alba* and *M. chamomilla*. The significant antibacterial activity against *S. aureus* was recorded for *M. chamomilla* (74.07%), *V. wallichii* (50.0%) and *O. basilicum* (40.74%) while no activity was found in the crude methanol extract of *C. alba* and *P. emblica*. In case of *X. compestris* considerable inhibitory potential was observed for *V. wallichii* (46.15%) and *M. chamomilla* (42.30%) while minimum potential was examined for *P. emblica* (36.53%) however *O. basilicum* was failed to show any inhibitory zone against the test bacterial strain. The standard drug Ciprofloaxcin was used as positive control showed significant inhibitory zone whereas pure dimethyl sulfoxide (DMSO) used as negative control don't exhibited bactericidal activity against all the test strains.

Table 4. Inhibitory zone (%) of crude methanol extract of selected plants against pathogenic bacterial isolates.

Plant species	Bacterial strains					
	A. T	B. S	C.M	E. C	S. A	X.C
C. alba	42.30	50.0	NA	NA	NA	38.46
M. chamomilla	69.23	53.57	59.25	NA	74.07	42.30
O. basilicum	NA	32.14	NA	54.0	40.74	NA
P. emblica	NA	NA	NA	48.0	NA	36.53
V. wallichii	54.38	NA	NA	56.0	50.0	46.15
Ciprofloxacin	26.0	28.0	27.0	25.0	27.0	26.0
DMSO	NA	NA	NA	NA	NA	NA

Antifungal activity

The results depicted in Table 5 reveals that considerable inhibitory zone against *A. flavus* was recorded for *C. alba* (52.38%) followed by *O. basilicum* (42.85%) and *V. wallichii* (38.09%) however *M. chamomilla* and *P. emblica* failed to showed antifungal activity. In case of *A. niger* promising zone of inhibition was observed for *V. wallichii* (68.42%) followed by *M. chamomilla* (39.47%) and *C. alba* (36.84%) while *O. basilicum* and *P. emblica* didn't exhibited any inhibitory diameter zone. Likewise, significant antifungal potential against *F. oxysporum* was noted for *P. emblica* (63.04%), *O. basilicum* (60.86%), *M.*

chamomilla (56.52%) and *V. wallichii* (52.17%) whereas the test strains was resistible to *C. alba*. The maximum inhibitory zone against *Paceilomyces sp.,* was recorded for *V. wallichii* (77.27%) and *P. emblica* (75.0%) as compared to *M. chamomilla* (50.0%) and *C. alba* (36.36%) whereas no activity was observed for *O. basilicum*. In case of *T. harzianum* highest inhibitory zone was examined for *C. alba* (52.0%) followed by *M. chamomilla* (48.0%) while *O. basilicum, P. emblica* and *V. wallichii* don't showed any inhibitory zone. The standard drugs itraconazole was used as positive control against *A. flavus* and *A. niger* whereas fluconazole was tested against *F. oxysporum, Paceilomyces sp.,* and *T. harzianum*. Both the drugs used against pathogenic fungal strains exhibited significant fungicidal activity and inhibited all the fungal strains.

Table 5. Inhibitory zone (%) of crude methanol extract of selected plants against pathogenic fungal isolates.

Plant species	Fungal isolates				
	A.F	A.N	F.O	Pac sp.	T.H
C. alba	52.38	36.84	NA	36.36	52.0
M. chamomilla	NA	39.47	56.52	50.0	48.0
O. basilicum	42.85	NA	60.86	NA	NA
P. emblica	NA	NA	63.04	75.0	NA
V. wallichii	38.09	68.42	52.17	77.27	NA
Fluconazole	NT	NT	23	22	25
Itraconazole	21	19	NT	NT	NT

A.F = *Aspergillus flavus,* A.N. *Aspergillus niger,* T.H = *Trichoderma harzianum, Pac sp. = Pacelomyces species* and F.H = *Fusarium oxysporum*.

Discussion

Proximate and nutrient analyses of edible plant and vegetables play a crucial role in assessing their nutritional significance. As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species [Ebrahimzadeh, M.A, 2010]. Fortunately, chemical composition diversity in plants also includes many compounds that are beneficial to humans such as vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value [Ebun-Oluwa, P.O, 2007]. The proportion of ash content reflects the mineral contents preserved in the food materials [Effiong, G.S.2009, Ene-obong, H. N. 1992]. The ash content determined in the present study was lower than the finding of [Ene-obong, H.N. 1992] who reported 17.1% ash content in *Eclipta alba*. The results of present study finds similar evidence from the data of [Exarchou, V,2002, Frazier, W.S,1978] who examined 10.61% and 12.90% of ash content in *C. siamea* and *Maerua angolensis*. Likewise [Gill, N.S, 2012] determined 8.53% of ash content in *Cassia nigricans* which justify the current data. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food [Gogoi, P, 2014]. High amount of moisture content proliferate microbial attack which causes spoilage [Gul, H, 2006]. The moisture content of any food is an index of its water activity and is used as a measure of stability and the susceptibility to microbial contamination [Gupta, R., 2016, Gupta, S.M, 2011]. The moisture content of selected plant species determined in the current study are in close proximity with [Hanif, R, 2006] who reported 8.33 g $100g^{-1}$ of moisture content for *Peperomia pellucida*. Crude fiber is made up largely of cellulose together with a little lignin which is indigestible in human [Hassan, L.G, 2007, Hausladen, A, 1999] reported that non-starchy vegetables are the richest sources of dietary fiber. Fiber helps in the maintenance of human health and has been known to reduce cholesterol level in the body [Hoffman, P.C, 1998]. A low fiber diet has been

associated with heart diseases, cancer of the colon and rectum, varicose veins, phlebitis, obesity, appendicitis, diabetes and even constipation [Ibanga, O.I, 2009, Jimoh F.O,2005]. Crude fiber enhances digestibility however its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage [Jones, M.M, 1985]. The fiber content observed during our research work are at par with the study of [Kalim, M.D, 2010] who reported 4.21% and 12.14% of crude fiber in *P. polyandra* and *X. aethiopica*. Therefore, the examined plants could be recommended as crude fiber source in the diet as a result of their relative high crude fiber content. Lipid provides very good sources of energy and aids in transport of fat-soluble vitamins, insulates and protects internal tissues and contributes to important cell processes [Krishnaraju, A.V, 2005, Lajide, L, 2008, Latif, A, 2003]. The crude fats observed in the present study are in line with the findings of [Lavanya, J., 2016]. Similar justification was also given by [Lee, Y.M, 2000] who recorded 6.70% and 3.12% of lipid content in *C. siamea* and *M. angolensis*. However, [Linder, M.C., 1991] noted 8.4% of crude lipids in the leaves of *Adansonia digitata* which also justify our results. The crude protein content examined during the current research work are also in close proximity with the findings of [Liu, X, 2008] who investigated 2.10 and 7.09 crude protein content in *Xylopia aethiopica* and *Parinari polyandra*. Likewise, [Liu, X., 2008.Mathew, S., 2006] observed 17.05% of crude protein in the leaves of *Aneilema aequinoctiale* which also supports our findings. [Mohanta, B., 2003] reported that diet is nutritionally satisfactory, if it contains high caloric value and a sufficient amount of protein. [Mubashir, S.,2014, Naidu, P.V.L.,2006, Nnamani, C.V., 2009] also stated that any plant foods that provide about 12% of their calorific value from protein are considered good source of protein. The NFE value calculated in our findings indicates that carbohydrates are the primary ingredient and the major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw materials for many industries [Oktyabrsky, O., 2009, Onwuka, G. I., 2005, Ooi, D., 2012].

Minerals are considered to be essential in human nutrition [Osman, M.A., 2004]. These minerals are vital for the overall mental physical well-being and are important constituent of bones, teeth, tissues, muscles, blood and nerve cells. They generally help in maintenance of acid-base balance, response of nerves to physiological stimulation and blood clotting [Pamela, C.C.,2005, Pandey, M.A.B.,2006]. Results of our study divulged that the examined plants contain different concentration of micronutrients. The data obtained in the present research work are at par with the findings of [Parekh, J., 2007, Park, S.K., 2014] who examined Cu, Mg, Zn, Fe, Cr, and Mn in *R. emodi*. [Park, S.K., 2014] reported Cu, Mg, Zn, Fe, Cr, and Mn from the rhizomes of *Rheum palmatum*. Likwise [Park, Y.S., 2008] observed Cd and Ca in the petioles of *Rheum rahbarbarum*. [Patil, D.D., 2011] evaluated C, O, Na, Mg, Al, Si, Cl, K, Ca and Fe in different parts of *V. odorata*. [Perez, C., 1990] reported C, O, Mg, Al, Si, K, Ca, Fe, S, Na, Cl, B, P and Zn in different parts of *Alstonia scholaris*. [Raghu, H.S., 2010, Rajurkar, N.S., 1997] reported chloride, nitrogen and potassium from *Nicotiana tabacum*.

Plants present to have a sufficient amount of free radical scavenger in the form of secondary metabolites like phenolics and flavonoids. They are present in each and every part of the plant [Said, S., 1996, Saldanha, L.G., 1995]. Flavonoids are an important plant secondary metabolite exhibit different biological property including anticancer, anti-allergic, antimicrobial and anti-inflammatory. They are also known as nature's biological response modifier because of their inherent ability to modify the body's reaction to allergies and virus [Scalbert, A., 2005. Scott, W.S., 1957]. The phenolic compounds have a considerable nutrition value in providing flavor, taste, color and aroma. They also contribute in the plant defense system by preventing molecular impairment, damage by insects, herbivorous and microorganism [Shinwari, Z.K., 2006]. It is the major group of phytochemicals in foods comprises of flavonoids, tannins, simple phenols, benzoic acid,

lignans, lignins, phenyl propanoids and stilbenes moiety. The current research work is at par with [Lajide, L., 2008] who reported similar results of total phenols content in *C. palustris* and *D. affinis*. The results of present study also finds supportive evidence from the study of [Singleton, V.L., 1965, Soare, L.C., 2012, Sofowora, A., 1982] who determined various concentration of total phenols and total flavonoid content in the examined plant species. Literature revealed that free radicals are the major cause of various chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [Sudhanshu, R, 2012] reported 55.58% of antioxidant activity in methanol fraction of *Caltha palustris* var. *alba* by using DPPH assay. Our results find supportive evidence from the studies of [Tairo, T.V., 2011] who examined that *C. obliqua* showed free radical scavenging ability against the DPPH radicals with IC_{50} <100 µg ml^{-1}. The free radical scavenging activity observed by DPPH assay indicate that methanol fraction of *M. chamomilla* had greater antioxidant activity when compared with ethanol extracts. [Thomas, T., 2011] Evaluated that ethanol extract obtained from the leaves of *O. basilicum* displayed the highest antioxidant activity due to removal of the stable radical DPPH as compared to CCl_4 extract of stem. Likewise, *P. emblica* obtained from Huizhou was found to have the strongest antioxidant activities in scavenging DPPH radicals, superoxide anion radicals, and had the highest reducing power. [Udgire, M.S., 2013] evaluated that methanol extract of *V. wallichii* has appreciable antioxidant activity.

In modern days, the antioxidants and antimicrobial activities of plant extracts have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy. These bioactive compounds of plants act as antioxidants by scavenging free radicals which also have healing capability of the disorders caused by free radical [Vaya, J., 1997]. The results of present study are at par with the findings of [Williamson, G., 1997] who investigated that *C. obliqua* have appreciable antimicrobial potential against *B. subtilis, S. aureus, S. epidermidis, E. coli, A. niger* and *Candida albicans*. Similar justification was also provided by [Zhang, X., 1976] who examined that extract obtained from the leaves of *D. cochleata* have significant antibacterial activity. [Sageratia thea,2006] reported that leaf extract of *Hippophae salicifolia* possess notable inhibitory activity against gram positive bacteria but in case of fungal cultures the seed extract showed significant activity against *Mucor indicus* and *Tilletia indica*.

The present results also find supportive evidence from the study of [Sulieman, M.M., 2008] who observed that essential oil and methanol extract of *M. chamomilla* have promising antimicrobial activity by using broth micro-dilution method. [Taesotikul, T., 2003] reported that ethanol extract *O. basilicum* exhibited a potent antibacterial activity against *E. coli, P. aeruginosa, P. mirabilis, K. pneumoniae, S. aureus* and *E. faecalis*. Likewise, [Tehrian, A.A., 2009] noted that methanol extract of *P. emblica* has significant antimicrobial activity against *K. pneumoniae* and *S. aureus*. Our results are also in line with [Tesema, S., 2015] who screened the rhizome and root extracts of *V. wallichii* against *S. aureus, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, A. niger, C. albicans* and *M. furfur* using agar diffusion method. It was observed that ethanol and methanol extracts showed significant antimicrobial activity ranging from 13-18mm diameter of zone of inhibition especially against fungal pathogens as compared to bacterial pathogens. The present results also find supportive evidence from the study of [Treas, G.E., 1985] who observed that essential oil and methanol extract of *M. chamomilla* have promising antimicrobial activity by using broth micro-dilution method.

References

Abdul-Latif FM, Mohammad N, Edou Ali AA, Djama SO, Obam L, Bassole IHN, Dicko MH. 2011. Antimicrobial and antioxidant activities of essential oil and methanol extract of *Matricaria chamomilla* L. from Djibouti. Journal of Medicinal Plant Research **5,** 1512-1517.

Abiy Y, Solmon D, Jacob DM, Christine CB, Matthias H, Martin GP. 2005. Antimicrobial flavonoids from the stem bark of *Erythrina burtii*. Fitoterapia. **96,** 496-499.

Abolaji OA, Adebayo AH, Odesanmi OS. 2007. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica, Blighia sapida* and *Parinari polyandra*) commonly used by pregnant women in the Western part of Nigeria. Pakistan Journal of Nutrition **6,** 665-668.

Adam AA, Omer AFA. 2015. Antibacterial activity of *Ocimum basilicum* (Rehan) leaf extract against bacterial pathogens in Sudan. American Journal of Research Communication **3,** 94-99.

Agostoni C, Riva R, Giovannini M. 1995. Dietary fiber in waning foods of young children. Pediatrics **96,** 1000-1005.

Aiyelaagbe OO, Osamudiamen PM. 2009. Phytochemical screening for active compounds in *Mangifera indica*. Plant Science Research **2,** 11-13.

Akpabio UD, Ikpe EE. 2013. Proximate composition and nutrient analysis of *Aneilema aequinoctiale* leaves. Asian Journal Plant Science Research **3,** 55-61.

Akpanyung EO, Udoh AP, Akpan EJ. 1995. Chemical composition of the edible leaves of *Pterocarpus mildbreadii*. Plant Foods Hum Nutrition **48,** 209-2015.

Ali A. 2010. A comparative study of nutrients and mineral molar ratios of some plant foods with recommended dietary allowances. Journal of Food Science Technology **2,** 104-108.

AOAC. 2012. Association of Official Analytical Chemists. Official Methods of Analysis 19th Edition (George Latimer.ed.). AOAC Intl, Arlington, Virginia, USA.

Ayo RG. 2013. Proximate and mineral compositions of the leaves and stem bark of *Cassia nigricans* Vahl. International Journal of Medicinal Plants Research **2,** 242-246.

Balandrin MF, Kjocke AJ, Wurtele E. 1985. Natural plant chemicals: sources of industrial and mechanical materials. Science **228,** 1154-1160.

Beaglehole R, Irwin A, Prentice T. 2004. The World health report 2004, changing history. Statistical Annex. Deaths by cause, sex and mortality stratum in WHO regions, estimates for 2002. WHO, Geneva, p 120-122.

Bello MO, Falade OS, Adewusi SRA, Olawore NO. 2008. Studies on the chemical compositions and antinutrients of some lesser known Nigeria fruits. African Journal of Biotechnology **7,** 3972-3979.

Bibi S, Dastagir G, Hussain F, Sanaullah P. 2006. Elemental composition of *Viola odorata* Linn. Pakistan Journal of Plant Science **12,** 141-143.

Blackwell WH. 1990. Poisonous and Medicinal Plants. Prentice Hall: Englewood Clifts, NJ.

Cheeke PR. 1998. Natural toxicants in Feeds, Forages, and Poisonous Plants, 2nd Edition. Interstate Pubs II.

Choudhary RK, Saroha AE, Swarnkar PL. 2011. Radical scavenging activity of phenolics and flavonoids in some medicinal plants of Indian Journal of Pharmacy Research. **4,** 712-713.

Chung KT, Weichang I, Johnson MJK. 1998. Are tannins a double edged sword in biology and health? Trends Food Science Technology **4,** 168-175.

Dastagir G, Pervez N. 2004. Elemental composition of *Alstonia scholaris* Linn. Pakistan Journal of Plant Science **10,** 47-50.

Davey KR. 1989. A predictive model for combined

temperature and water activity on microbial growth during the growth phase. Journal of Applied Microbiology **65,** 483-488.

Desai BB, Salunkhe DK. 1991. Fruits and vegetables. In: Salunkhe DK, Desh-pande SS (Eds). Foods of Plant Origin. Production, Technology and Human Nutrition, AVI, New York, p 301-355.

Dingman SL. 2002. Water in soils: infiltration and redistribution. Physical hydrology, 2nd edition, upper saddle river, New Jersey: Prentice-Hall, Inc. p 646.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Pourmorad F. 2010. Nitric oxide radical scavenging potential of some Elburz medicinal plants. African Journal of Biotechnology **9,** 5212-5217.

Ebun-Oluwa PO, Alade AS. 2007. Nutritional potential of berlandier nettle spurge (*Jatropha cathatica*) seed. Pakistan Journal of Nutrition **6,** 345-348.

Effiong GS, Ibia TO, Udofia US. 2009. Nutritive and energy values of some wild fruit spices in Southeastern Nigeria. Journal of Environmental Agriculture Food Chemistry **8,** 917-923.

Ene-obong HN. 1992. Nutritional evaluation, composition pattern and processing of underutilized traditional foods with particular reference to the African yam beans (*Sphenostylis stenocarpa*). PhD thesis, Department of Home Science and Nutrition, University of Nigeria, Nsukka.

Exarchou V, Nenadis U, Tsimidou U, Gerothanassis IP, Troganis A, Boskou D. 2002. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage and summer savory. Journal of Agriculture Food Chemistry **50,** 5294-5299.

Frazier WS, Westoff DC. 1978. Food Microbiology. 3rd Edition, McGraw Hill, New York, p 278-298.

Gill NS, Sharma R, Arora R, Bali M. 2012. Antioxidant and antibacterial activity of *Hippophae rhamonides* methanolic leaf extracts from dry temperate Agro-climate region of Himachal Pradesh. Journal of Plant Science **7,** 194-200.

Gogoi P, Kalita JC. 2014. Proximate analysis and mineral components of some edible medicinally important leafy vegetables of Kamrup district of Assam, India. International Journal of Pharmacy Biological Science **5,** 451-457.

Gul H, Khattak RA, Muhammad D. 2006. Yield and chemical composition of tobacco leaves of different cultivars as effected by four levels of potassium chloride. Pakistan Journal of Science and Industrial Research **49,** 125-133.

Gupta R, Gupta GD. 2016. Pharmacognostic, phytochemical and antioxidant activity studies on *Cordia obliqua* willd. Leaf. International Journal of Pharm Science Research **7,** 4669-4677.

Gupta SM, Gupta AK, Ahmed Z, Kumar A. 2011. Antibacterial and Antifungal Activity in Leaf, Seed Extract and Seed Oil of Seabuckthorn (*Hippophae salicifolia* D. Don) Plant. Journal of Plant Pathology and Microbiology **2,** 1-4.

Hanif R, Iqbal Z, Iqbal M, Hanif S, Rasheed M. 2006. Use of vegetables as nutritional food: Role in human health. Journal of Agriculture Biological Science **1,** 18-20.

Hassan LG, Ngaski MMA. 2007. Nutritional evaluation of *Cassia siamea* leaves. Journal of Chemical Society **32,**137-143.

Hausladen A, Stamler JS. 1999. Nitrosative stress. Method Enzymology **300,** 389-395.

Hoffman PC, Comb DK, Casler MD. 1998. Performance of lactating dairy cows fed alfalfa silage

or perennial ryegrass silage. Journal Dairy Science **81,** 162-168.

Ibanga OI, Okon DE. 2009. Minerals and anti-nutrients in two varieties of African pear (*Dacryodes edulis*). Journal of Food Technology **7,** 106-110.

Jimoh FO, Oladiji AT. 2005. Preliminary Studies on *Piliostigma thonningii* seeds: Proximate analysis, mineral composition and phytochemical screening. African Journal of Biotechnology **4,** 1439-1442.

Jones MM, Johnson DO, Netlerville JT, Wood JI, Joesten MD. 1985. Chemistry and Society. 5th Edition Saunders College Publishers USA, p 521- 577.

Kalim MD, Bhattacharyya D, Banerjee A, Chattopadhyay S. 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Complementary Alternative Medicine **10,** 1-11.

Krishnaraju AV, Rao TV, Sundararaju D. 2005. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemisia salina*) lethality assay. International Journal of Applied Science Engineering **2,** 125-134.

Lajide L, Oseke MO, Olaoye OO. 2008. Vitamin C, fibre, lignin and mineral contents of some edible legume seedlings. Journal of Food Technology **6,** 237-241.

Latif A, AhmadH, Begum S, Adnan M, Hussian S, Waseem S. 2003. Medicinal and other economic plants as substitute to forest logging in Miandam and Sulatanr valleys, Swat. Proceedings of international workshop on conservation and sustainable use of medicinal and aromatic plants in Pakistan. WWF-Pak. p 101-105.

Lavanya J, Selvam SP, Priya MJ, Jacintha P, Aradana M. 2016. Antioxidant and antimicrobial activity of selected medicinal plants against human oral pathogens. International Journal of Pharma Science **8,** 71-78.

Lee, YM, Kim H, Hong JK, Kang BH, Kim SJ. 2000. Water extract of 1:1 mixture of *Phellodendron cortex* and *Aralia cortex* has inhibitory effects on oxidative stress in kidney of diabetic rats. Journal of Ethno pharmacology **73,** 429-436.

Linder MC. 1991. Nutrition and metabolism of the major minerals, in: Linder MC (Ed.) Nutrition biochemistry and metabolism with clinical applications, 2nd Edition. Appleton and Lange, USA.

Liu X, Zhao M, Wang J, Yang B, Jiang Y. 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of Food Computational analysis **21,** 219-228.

Mathew S, Abraham TE. 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. Food Chemistry **94,** 520-528.

Mohanta B, Chakraborty A, Sudarshan M, Dutta RK, Baruah M. 2003. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. Journal of Radio analytical Nuclear Chemistry **258,** 175-179.

Mubashir S, Dar MY, Lone BA, Zargar MI, Shah WA. 2014. Anthelmintic, antimicrobial, antioxidant and cytotoxic activity of *Caltha palustris* var. alba Kashmir, India. Chinese Journal of Natural Medicine **12,** 567-572.

Naidu PVL, Kumar KK, Kumar KM, Gunesh G, Narasimha MR. 2006. Antimicrobial activity of *Achyranthes aspera*. Biosciences, Biotechnology Research Asia **03,** 1.

Nnamani CV, Oselebe HO, Agbatutu A. 2009. Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi

State, Nigeria. African Journal Biotechnology **8,** 2321-2324.

Oktyabrsky O, Vysochina G, Muzyka G, Samoilova Z, Kukushkina T, Smirnova G. 2009**.** Assessment of anti-oxidant activity of plant extracts using microbial test systems. Journal of Applied Microbiology **106,** 1175-1183.

Onwuka GI. 2005. Food Analysis and Instrumentation; Theory and Practice. Naphthalic prints, Surulere, Lagos, Nigeria, p 219- 230.

Ooi D, Iqbal S, Ismail M. 2012. Proximate composition, nutritional attributes and mineral composition of *Peperomia pellucida* L. (Ketumpangan Air) grown in Malaysia. Molecules. **17,** 11139-11145.

Osman MA. 2004. Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed protein solubility. Plant Foods Human Nutrition **59,** 29-33.

Pamela CC, Richard AH, Denise RF. 2005. Lippincotts illustrated Reviews Biochemistry 3rd Edition, Lippincott Williams and Wilkins, Philadelphia, p 335- 388.

Pandey MAB, Abidi S, Singh RP. 2006. Nutritional evaluation of leafy vegetables. Paratha. Journal of Human Ecology **19,** 155-156.

Parekh J, Chanda SV. 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology **31,** 53-58.

Park SK, Choi CW, Lee YK, Hyun PJ, Arokiyaraj S. 2014. Antimicrobial and free radical scavenging activity of *Cordia obliqua* W. (*Boraginaceae*) fruit extracts. Journal of Pure Applied Microbiology **8,** 117-120.

Park, YS, S, Jung SG, Kang Heo BK, Arancibia-Avila P, Toledo F. 2008. Antioxidants and proteins in ethylene treated kiwifruits. Food Chemistry **107,** 640-648.

Patil DD, Mhaske DK, Wadhawa GC. 2011. Antibacterial and antioxidant study of *Ocimum basilicum* Labiatae (sweet basil). Journal of Advance Pharmacy and Educational Research **2,** 104-112.

Perez C, Pauli M, Bazevque P. 1990. An antibiotic assay by the agar well diffusion method. Acta Biology Medical Expression **15,** 113-115.

Raghu HS, Ravindra P. 2010. Antimicrobial activity and phytochemical study of *Phyllanthus emblica* linn. International Journal of Pharmacy Studies Research **1,** 30-33.

Rajurkar NS, Damame MM. 1997. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. Journal of Radio analytical Nuclear Chemistry **219,** 77-80.

Said S, Saeed HMA, DeSilva LA, Zubairy HN, Bano Z. 1996. Medicinal Herbal, A Textbook for Medical Students and Doctors. Published by Hamdard Foundation Pakistan Nazimabad, Karachi 74600, Pakistan **1,** 272-291.

Saldanha LG. 1995. Fiber in the diet of U.S. Children: Results of national survey. Pediatrics **96,** 994-996.

Scalbert A, Manach C, Remesy C, Morand C. 2005. Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science Nutrition **45,** 287-306.

Scott WS. 1957. Water relations of food spoilage microorganisms. Advance Food Research **7,** 84-127.

Shinwari ZK, Rehman M, Watanabe T, Yoshikawa T. 2006. A Pictorial guide to medicinal plants of Pakistan. Kohat University of Science and Technology Pakistan.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology Viticulture **16,** 144-158.

Soare LC, Ferdes M, Stefanov S, Denkova Z, Nicolova R, Denev P, Bejan C, Paunescu A. 2012. Antioxidant activity, polyphenols content and antimicrobial activity of several native pteridophytes of Romania Liliana Cristina. Not. Botanical Horticulture Agrobiology **40,** 53-57.

Sofowora A. 1982. Medicinal plants and traditional medicine in Africa. Wiley, Nigeria, p 33-34.

Sudhanshu Rao N, Mittal S, Menghani E. 2012. Evaluation of antioxidant properties of Valeriana wallichi to scavenge free radicals. Asian Journal of Pharmacology Clinical Research **5,** 238-240.

Tairo TV, Jesse TN, Lukhoba WC, Herbert VML. 2011. Nutritive and antinutritive qualities of mostly preferred edible woody plants in selected dry lands of Iringa District, Tanzania. Pakistan Journal of Nutrition **10,** 786-791.

Thomas T. 2011. Preliminary antibacterial and phytochemical evaluation of *Dryopteris cochleata* (D. Don) Global Pharm Technology **3,** 4-8.

Sulieman MM, Dzenda T, Sani CA. 2008. Antidiarreal activity of the methanol stem-bark extract of *Annona senagalensis.* Journal of Ethnopharmacology **116,** 125-130.

Taesotikul T, Panthong A, Kanjanapothi D, Verpoorti R, Scheffer JJC. 2003. Anti-inflammatory, antipyretic and antinoceceptive activities of *Tabernaemontena pandacaque* poir. Journal of Ethnopharmacology **84,** 31-35.

Tehrian AA, Babaei M, Vafaei AA, Jarrahi M, Jadidi M, Sadeghai H. 2009. Antinoceceptive effect of Hydrochloric extracts of *Thymus vulgaris*. Pak Journal of Pharmacological science **22(1),** 83-89.

Tesema S, Makonnen E. 2015. In vivo analgesic and antipyretic activities of N-Butanol and water reactions of *Ocimum suave* aqueous leaves extracts in mice. Ethiop Journal of health and science **25(2),** 139-146.

Treas GE, Evans WC. 1985. Textbook of Pharmacognosy. Published by Balleire Tindal, London.

Udgire MS, Pathade GR. 2013. Evaluation of antimicrobial activities and phytochemical constituents of extracts of *Valeriana wallichii*. Asian Journal of Plant Science Research **3,** 55-59.

Vaya J, Belinky GA, Aviram M. 1997. Antioxidant constituents from Licorice roots: isolation, structure elucidation and anti-oxidative capacity toward LDL oxidation. Free Radical Bio Medical **23,** 302-313.

Williamson G, Dupont MS, Heaney RK, Roger G, Rhodes MJ. 1997. Induction of slutathione S transferase activity in hepG2 cells by extracts of fruits and vegetables. Food Chemistry **2,** 157-160.

Zhang X. 1976. Traditional medicine and WHO, World health, the magazine of World Health Organization 49th year **2,** 4-5.