The Journal of Animal & Plant Sciences, 30(2): 2020, Page: 454-460
ISSN: 1018-7081

PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST *ALTERNARIA* SPECIE ISOLATED FROM ONION

R. Kalsoom[1,2*], M. S. Haider[1] and S. Chohan[2*].

[1]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
[2]Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan.
Corresponding author Email: rabia.iags@gmail.com, sobiachohan@bzu.edu.pk

ABSTRACT

Medicinal plants are the immense source of bioactive compounds and can be used for the treatment of various diseases of plants, animals and even human beings. Aim of the present study was to investigate the antifungal potency of leaf extracts of *Citrus limon*, *Cassia fistula* and *Euclaptus camaldulensis* against *Alternaria* specie associated with onion leaves through poisoned food technique. Based on the outcome of our results, methanolic leaf extracts of *C. limon* showed highest inhibition (61%) followed by *C. fistula* (53%) and *E. camaldulensis* (44%) respectively. Phytochemical analysis of best result oriented citrus extract was done with Gas Chromatography-Mass Spectrometry (GC-MS) technique. The results revealed the presence of antimicrobial compounds predominantly hexacosane (21.41%), delta-tocopherol (13.25%), gamma-tocopherol (9.61%), valproic acid (8.24%), gamma-sitosterol (3.98%), cetene (3.84%), isoquinoline,1-[(3,4-diethoxyphenyl)methyl]-6,7-diethoxy- (3.36%), nonadecanoic acid, methyl ester (3.05%), benzeneacetic acid (2.95%). Present study indicates that plant extracts could be a potential alternative in developing a potent plant-based fungicide which can be used in organic farming for the management of diseases in plants. Therefore, extensive research is required to detect more compounds in order to develop effective management approaches that significantly reduce the impact of the pathogens on human health as well as on environment.

Key words: Antifungal potential; poisoned food technique; biologically active compounds; GC-MS analysis; *Citrus limon.*

https://doi.org/10.36899/JAPS.2020.2.0039 *Published online March 02, 2020*

INTRODUCTION

From the ages, medicines made from the plants were used against various diseases. Usage of medicinal plant extracts became popular in late 1990s and still now a day, they are playing vital role in discovering the new drugs specially in developing countries. In nature medicinal plants are rich source of the antimicrobial compounds which can be effective against pathogens. Pakistan has blessed with large number of medicinal plants rich in anti-microbial compounds those possess antimicrobial activities. These plants are being used in preparation of various drugs since decades (Javed *et al*., 2019; Riaz *et al*., 2019).

Plant diseases can be checked completely using synthetic fungicides but due to residual toxicity, environmental pollution and development of resistance in the pathogens it is essential to improve the treatment methods so that these problems can be coped (Fankam *et al*., 2014). Therefore, it is need of the day not only to improve the existing drugs but also discover the new effective ones. Many studies have proved that medicinal plants are having diverse antifungal and antibiotic properties. Extracts of medicinal plants are not only cost effective and has negligible side effect on environment but also minimize the health hazards of synthetic chemicals or fungicides (Pu *et al.,* 2017; El-Shouny *et al.,* 2018). According to the WHO (World Health Organization) 70–80% of the population relay on herb plants in the world (Muhammad *et al.,* 2011). There is a list of compounds such as phenolics, alkaloids, flavonoids, and terpenoids which make them effective and open the new horizons to discover the novel complexes against the pathogens which leads towards new drug development (Kumar *et al.,* 2016; Nayak *et al.,* 2017; Ahmed *et al.,* 2018; Al-Tohamy *et al.,* 2018). *C. limon* (*Rutaceae*), *C. fistula* (*Caesalpiniaceae*) and *E. camaldulensis* (*Myrtaceae*) are diversified groups of medicinal plants, well known for its pesticidal and antifungal effects and can be used in treatment of different diseases of plants, animals and humans (Gupta *et al.,* 2000; Abdel-Hafez *et al.,* 2013; Siddiqua *et al*., 2018).

Onion (*Allium cepa*) is an everyday used crop and grown all over the world. It is a rich source of dietary fiber, energy, vitamins (A and C) and minerals such as calcium, manganese and iron (Priya *et al.,* 2015; Pareek *et al.,* 2017). More than 66 diseases attack on the onion plant at different growth stages. Purple blotch of onion caused by *Alternaria* sp. is among the drastic diseases and attacks on every onion growing pocket of the world. According to Maini *et al.* (1985) 5 to 50% losses are recorded due to this disease in all over the world. Now a day's exploration of neglected plants as a potent source of alternative biomedicine become a hot issue. In our study, we assessed the effectiveness of three native tree leaves such as *C. limon*, *C. fistula* and *E. camaldulensis* against *Alternaria* sp. isolated from diseased onion leaves. Moreover, different compounds present in citrus plant were determined through GC-MS analysis.

MATERIALS AND METHODS

Preparation of plant extracts: Leaves of three medicinal plants (*C. limon, C. fistula* and *E. camaldulensis.*) were collected from the Multan region, washed with tap water, shade dried and then homogenized into fine powder. This fine powder was used to make the methanolic extract of these plants according to the method described by Rafiq *et al.* (2017). Twenty-five grams of each plant powder was dipped into 100 ml of methanol for 10 days. After that, extracts were passed through muslin cloth, filtered and dried at 45 ℃ on hot water bath. The resultant pastes were stored in brown bottles at 4 ℃ till use.

Procurement of fungus: Diseased onion leaves (*Allium cepa*) showing typical purple blotch symptoms were collected from onion field. The fungus was isolated and purified from the diseased leaves on potato dextrose agar medium (PDA) according to the standard procedures. The isolated fungus was identified based on morphological characteristics as suggested by Ellis, (1971).

Antifungal effect of plant extracts: Different concentrations (100%, 75%, 50%, 25%, 15%, 5%) of plant extracts were prepared by dissolving the resultant extract paste in Dimethyl sulfoxide (DMSO). They were used against isolated fungus by food poisoned technique. Plant extracts were mixed in PDA medium were poured into sterilized petri plates and mycelial disc (6mm) was placed on the center of solidified plate. Plates without plant extracts (having only DMSO) served as negative control. Recommended dose (6g/L) of Mancozeb was kept as positive control. Each treatment was replicated thrice. Plates were incubated for 15days at 28± 2 ^{0}C at alternate light and dark condition. The radial growth of fungal colony was measured on alternate days and percentage inhibition was calculated by this formula of Vincent (1947):

$I = (C - T)/C \times 100$

Where 'I' is percentage growth inhibition, "C' is mean diameter (cm) of fungal colony in control and "T" is mean diameter (cm) of fungal colony with plant extract.

GC-MS analysis of plant extracts: GC-MS analysis of methanolic extracts of citrus showing most promising antifungal activity was carried out by using an Agilent 7890C gas chromatograph in tandem with a 5975C MSD and HP5MSI separation column whose length was 30 m, ID was 0.250 mm and its film thickness was 0.25 µm. Identification and quantification of compounds ware conducted using AMDIS with a manually curated retention indexed GC-MS library while additional identification of compounds was performed using the NIST17 and Wiley 11 GC-MS spectral libraries.

Statistical analysis: One-way ANOVA was applied by using statistics 8.1 software program. Mean and standard errors were calculated for three replicates. Means were compared by the LSD tests at significant level (≤ 5%).

RESULTS

Antifungal activity of plant extracts: The antifungal activity of different plant leaf extracts (*C. limon*, *C. fistula* and *E. camaldulensis*) were investigated by using food poisoned technique against purple blotch disease of onion. The results indicated that all plant extracts have shown good antifungal activities as compared to the negative control while mancozeb gave complete inhibition. Among the tested plants *C. limon*, performed best as compare to *C. fistula* and *E. globulus.* Different concentrations of methanolic leaf extracts of *C. limon* from 5 to 100% significantly reduced fungus growth by 34.35-60.62% respectively while in case of *C. fistula* the reduction value is from 19.20 to 52.92 % and *E. camaldulensis* gave inhibition value from 14.18 to 44.29 % over the negative control (Fig. 1).

GC-MS analysis: Superlative result-oriented extract of *C. limon* was further subjected to GC-MS analysis (Fig 2). The compounds identified from the extract along with their molecular weight, molecular formulae, retention time and peak area (%) are presented in Table 1. Among them dominant compounds which share more peak area are enlisted with their nature and biological activity in Table 2.

The dominant compounds present in methanolic extracts in *C. limon* were hexacosane (21.410 %), delta-tocopherol (13.252%), gamma-tocopherol (9.614%), valproic acid (8.241%), gamma-sitosterol (3.98%), cetene (3.840%), isoquinoline, 1-[(3,4-diethoxyphenyl)methyl]-6,7-diethoxy- (3.36%), nonadecanoic acid, methyl ester (3.052%), benzeneacetic acid (2.951%), (2S)-1-Hydroxy-3-(palmitoyloxy)-2-propanyl (9Z)-9-hexadecenoate (2.136%), octacosane (2.018%), niacin, (1.975%), octadecanoic acid (1.834%), phloretic acid (1.640%), 2,2,5,5-tetramethyl-4-ethyl-3-imidazoline-1-oxyl (1.612%), pentanedioic acid, 3-methyl-3-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester (1.474%), methyl tetradecanoate (1.346%), l-isoleucine, N-trifluoroacetyl-(0.999%), 9,12-octadecadienoic acid (Z,Z) (0.978%). Different phenolic compounds were detected from citrus leaves which proved their medicinal value.

These phenolics are widely used as antimicrobial agent and their presence in the plant make it resistant.

Table 1. Compounds identified from methanolic inflorescence extract of *Citrus limon* through GC-MS analysis.

Sr. No.	Names of compounds	Formula	Mol. Wt.	Retention time (min)	Peak area (%)
1	Hexacosane	$C_{26}H_{54}$	366.42	13.76	21.410
2	delta.-Tocopherol	$C_{27}H_{46}O_2$	402.65	16.86	13.252
3	gamma.-Tocopherol	$C_{29}H_{50}O_2$	430.71	17.33	9.614
4	Valproic acid	$C_8H_{16}O_2$	144.21	4.61	8.241
5	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.39	19.41	3.986
6	Cetene	$C_{16}H_{32}$	224.25	8.98	3.840
7	Isoquinoline, 1-[(3,4-diethoxyphenyl)methyl]-6,7-diethoxy-	$C_{24}H_{29}NO_4$	395.21	13.37	3.363
8	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312.30	13.29	3.052
9	Benzeneacetic acid	$C_8H_8O_2$	136.15	6.52	2.951
10	(2S)-1-Hydroxy-3-(palmitoyloxy)-2-propanyl (9Z)-9-hexadecenoate	$C_{35}H_{66}O_5$	566.9	19.23	2.136
11	Octacosane	$C_{28}H_{58}$	394.45	16.28	2.018
12	Niacin	$C_6H_5NO_2$	123.11	6.48	1.975
13	Octadecanoic acid	$C_{18}H_{36}O_2$	284.27	12.90	1.834
14	Phloretic acid,	$C_9H_{10}O_3$	166.17	10.33	1.640
15	2,2,5,5-Tetramethyl-4-ethyl-3-imidazoline-1-oxyl	$C_9H_{17}N_2O$	169.13	4.87	1.612
16	Pentanedioic acid, 3-methyl-3-	$C_6H_{10}O_5$	162.17	9.15	1.474
17	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.22	9.99	1.346
18	l-Isoleucine, N-trifluoroacetyl-	$C_8H_{12}F_3NO_3$	227.08	6.49	0.999
19	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.24	12.78	0.978

Table 2. Major compounds identified from methanolic extract of *Citrus limon* through GC-MS analysis with their nature and biological activity.

No.	Name	Nature	Biological Activity	Reference
1.	Hexacosane	Acyclic alkanes	Anti-microbial, anti-bacterial.	Yasin *et al.* (2019)
2	delta.-Tocopherol	Phenolic	Anti- fungal, anti- bacterial, Anti-oxidants	Arulmozhi *et al.* (2018)
3	ᵞ-Tocopherol	Phenolic	Anti-tumor, Anti-oxidant, Anti-microbial	Kalita *et al.* (2018)
4	Valproic acid	long chain fatty acid.	Antiepileptic	Majnooni *et al.* (2016)
5	γ-Sitosterol	Steroids	Antimicrobial	Rafiq *et al.* (2017).
6	Cetene or 1-Hexadecene	aliphatic compound, long chain fatty acid.	Antibacterial, antifungal, antioxidant, anti-inflammatory,	Senthil *et al.* (2016); Egbung *et al.* (2017)
7	Isoquinoline, 1-[(3,4 diethoxyphenyl)methyl]-6,7-diethoxy-	alkaloid,	antimicrobial and anti-inflammatory	Gopalakrishnan and Rajameena (2012)
8	Nonadecanoic acid, methyl ester	fatty acids	Anti-inflammatory, anti-acne, insecticide.	Omoregie *et al.* (2018)
9	Benzeneacetic acid,	Phenols	antibacterial, antifungal, antioxidant, anticancer.	Rafiq *et al.* (2017); Valsalam *et al.* (2018)
10	(2S)-1-Hydroxy-3-(palmitoyloxy)-2-propanyl (9Z)-9-hexadecenoate	Aldehyde	Not Known	
11	Octacosane	acyclic alkanes	Mosquitocidal, antibacterial, antifungal activity.	Ghosh *et al.* (2012); Khatua *et al.* (2016)
12	Niacin	Carboxylic acid	Anticancer.	Jacobson *et al.* (1995)
13	Octadecanoic acid		Antioxidant, nematicide, Anti-inflammatory, Anti-cancer, Antimicrobial activities	Hema *et al.* (2011)
14	Phloretic acid	Polyphenols	Antioxidant, antimicrobial,	Kogiannou *et al.*

			anticancer, reduce cardiovascular diseases.	(2013); Ahmad *et al.* (2016).
15	2,2,5,5-Tetramethyl-4-ethyl-3-imidazoline-1-oxyl	Phenolics	Not reported	
16	Pentanedioic acid, 3-methyl-3-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	steroidal saponins	anti-inflammatory	Hong *et al.* (2012); Thi *et al.* (2018).
17	Methyl tetradecanoate	Myristic acid ester	Antioxidant, Anti-Cancerous Hypercholesterolemic, Nematicide activities.	Elaiyaraja and Chandramohan (2016).
18	l-Isoleucine, N-trifluoroacetyl-	Long branched amino acid	Antitumor	Wang *et al.* (2015)
19	9,12-Octadecadienoic acid (Z,Z)-	Fatty acid ester	Antioxidant, Nematicide, Anti-inflammatory, Antimicrobial, antiarthritic.	Elaiyaraja and Chandramohan (2016)

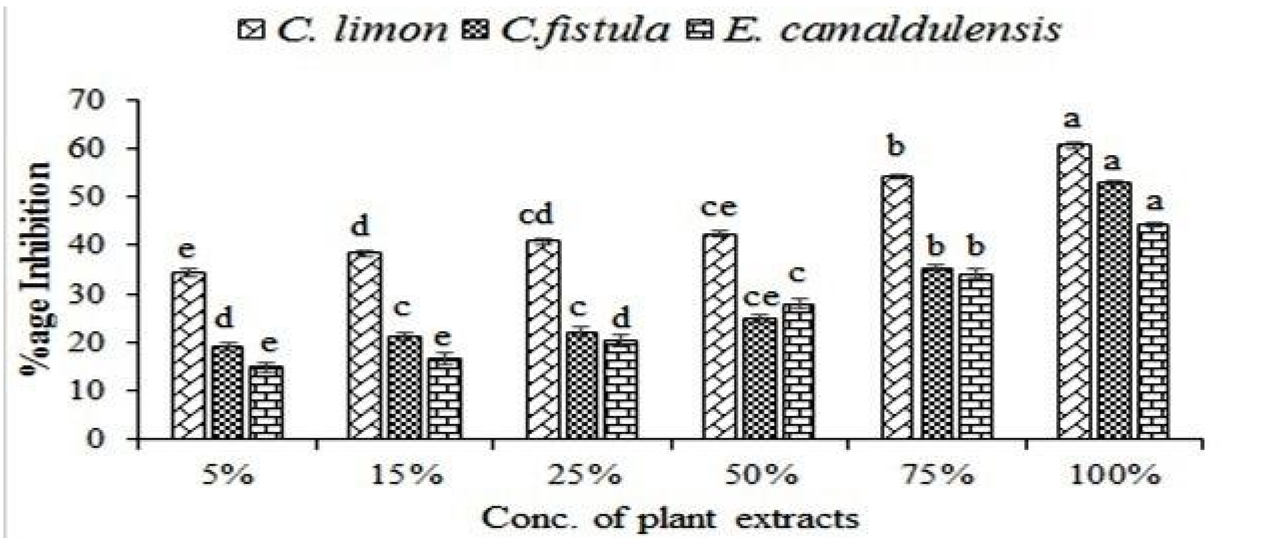

Fig. 1. Antifungal activity of tested plant extracts at different concentrations.

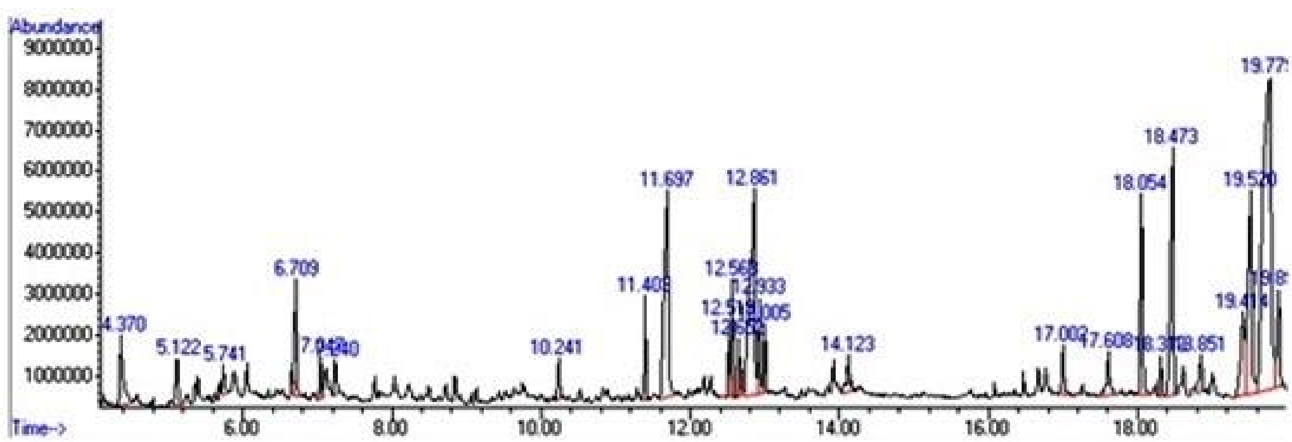

Fig. 2. GC-MS chromatograms of methanolic extracts of *Citrus limon*.

DISCUSSION

Medicinal plants are rich in natural antimicrobial compounds and play a vital role in development of new of drugs. In literature antimicrobial properties of these plants are reported against many human and plant pathogens (Arulmozhi *et al.*, 2018; Vijayalakshmi *et al.*, 2019) but are difficult to evaluate due to choose of solvents used, method of extraction, microbes used and procedures of antimicrobial test. In the present study, antimicrobial activities of all extracts of *C. limon*, *C. fistula* and *E. camaldulensis* were used against *Alternaria* sp. isolated from purple blotched onion leaves. Dry leaves of these plants were extracted by using the methanol as a solvent. Nearly all antimicrobial compounds of plants are either aromatic or saturated organic in nature, so they were easily extracted by using ethanol or methanol. It also has been known that different solvents have different solubility capacities for diverse phytochemicals (Cowan, 1999).

All the tested extracts showed antifungal activity against isolated fungus. Among them, leaf extract of *C. limon* showed maximum inhibition (60.62%) while *C. fistula* and *E. camaldulensis* showed inhibition of 52.92 % and 44.29% respectively. Mishra *et al.* (2008) evaluated leaf extracts of *C. limon* and *C. fistula* against *A. porri* and *Stemphylium* sp. and observed 72% and 62% of growth inhibition respectively. However, Abdel-Hafez *et al*. (2013) indicated that *Eucalyptus* leaf extracts (500µl) formed maximum zone inhibition (8.6 mm) against *A. porri.* while Meena, (2012) observed that *Eucalyptus globulus* at 5% conc. gave 92.64% inhibition against *A. porri*.

Different concentrations of plant extracts were used in present experiment. The results indicated that as the concentration of plant extract increased, it became more toxic towards fungus. This is not only true in case of *C. limon* whose percent inhibition increased ranging from 33.66 to 63.27% but also in case of *C. fistula* and *E. camaldulensis* as their fungal inhibition percentage was increased from 18.16 to 51.76 % and 20.99 to 45.31% respectively, when their concentration doses were increased. (Abdel-Hafez *et al.,* 2013; Gaikwad *et al.,* 2014).

The antimicrobial activity of extracts is due to presence of different chemicals such as steroids, alkaloids, flavonoids, triterpenoids and phenolic compounds or free hydroxyl groups. The presence and active release of these secondary compounds is usually responsible for biocontrol as they may interfere in enzyme system, permeability of cell membrane, synthesis of vital structures and in energy production (Tshabalala *et al.*, 2019; Vijayakumar *et al.*, 2019). GC-MS analysis is a potential means for identification of compounds present in the plant extracts. In this study, nineteen major compounds were identified from the methanolic leaf extract of *C. limon* while fifty other compounds shared minor peek area. Pandian and Noora, (2019) observed sixteen phytochemicals present in the methanolic leaf extracts of *Citrus medica* while Hojjati and Barzegar, (2017) identified twenty-seven compounds including linalool, geraniol, α-terpineol and linalyl acetate. The biological activity of *C. limon* documented by different scientists is elaborated in Table 2.

Conclusion: Present study indicated that traditional use of medicinal plants was effective for antimicrobial activities. It is concluded that methanolic leaf extract of *C. limon* showed potential antifungal activity against *Alternaria* sp. associated with blotched leaves of onion, might be due to presence of bioactive compounds. Thus, the extensive research is required to detect more compounds for formation of stable products in order to develop effective management approaches that significantly reduce the impact of the pathogens on human health as well as on environment.

Conflict of Interest: The authors declare that they have no conflict of interest.

Acknowledgement: This work was supported by the Higher Education Commission (HEC) Pakistan under the Indigenous Scholarship Program, Batch-III Phase II and we also acknowledge W. M. Keck Metabolomics Research Laboratory, Iowa State University, USA for GC-MS analysis.

REFERENCES

Abdel-Hafez, S., K. Abo-Elyousr, and I. Abdel-Rahim (2013). Effect of certain plant extracts to control purple blotch disease of onion plants (*Allium cepa* L.). J Plant Physiol Pathol 4: 1-4.

Ahmad, N., Y. Zuo, X. Lu, F. Anwar, and S. Hameed (2016). Characterization of free and conjugated phenolic compounds in fruits of selected wild plants. Food Chem. 190:80-89.

Ahmed, S., G. Kaur, P. Sharma, S. Singh, and S. Ikram (2018). Fruit waste (peel) as bio-reductant to synthesize silver nanoparticles with antimicrobial, antioxidant and cytotoxic activities. J. Appl. Biomed. 16(3): 221-231.

Al-Tohamy, R., S.S. Ali, K. Saad-Allah, M. Fareed, A. Ali, A. El-Badry, N.A. El-Zawawy, J. Wu, J. Sun, and G.H. Mao (2018). Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from Egyptian flora. J. Appl. Biomed. 16(4): 289-300.

Arulmozhi, P., S. Vijayakumar, and T. Kumar (2018). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. Microb Pathog. 123:219-226.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin Microbiol Rev 12(4): 564-582.

Egbung, G.E., C. Anosike, A.B. Utu-Baku, I. Ogar, and V.U. Nna (2017). Phytochemical evaluation and GC-MS analysis of *Hyptis verticillata* cultivated in Calabar Cross River State, Nigeria. IJBCS. 11(5): 2548-2559.

Elaiyaraja, A., and G. Chandramohan (2016). Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. J Pharmacogn Phytochem. 5(6): 158-171.

Ellis, M.B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England.

El-Shouny, W.A., S.S. Ali, J. Sun, S.M. Samy, and A. Ali (2018). Drug resistance profile and molecular characterization of extended spectrum beta-lactamase (ESβL)-producing *Pseudomonas aeruginosa* isolated from burn wound infections. Essential oils and their potential for utilization. Microb. Pathog. 116: 301-312.

Fankam, A.G., J.R. Kuiate, and V. Kuete (2014). Antibacterial activities of Beilschmiedia obscura and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. BMC Complement Altern Med. 14(1): 241.

Gaikwad, K., S.U. Jadhav, and V. Kakulte (2014). Management of fungal diseases of onion (Allium cepa L.) by using plant extract. Int. J. Life Sci. Pharm. Res. 4 (2): 28-30.

Ghosh, A., N. Chowdhury, and G. Chandra (2012). Plant extracts as potential mosquito larvicides. Indian J. Med. Res. 135(5): 581-598.

Gopalakrishnan, S., and R. Rajameena (2012). GC-MS analysis of some bioactive constituents of the leaves of *Desmodium gyrans* DC. Int. Res. J. Pharm. 3(8): 271-274.

Gupta, M., U. Mazumder, N. Rath, and D. Mukhopadhyay (2000). Antitumor activity of methanolic extract of *Cassia fistula* L. seed against *Ehrlich ascites carcinoma.* J. Ethnopharmacol.72(1-2): 151-156.

Hema, R., S. Kumaravel, and K. Alagusundaram (2011). GC/MS determination of bioactive components of *Murraya koenigii*. Am. J. Sci. 7(1): 80-83.

Hojjati, M., and H. Barzegar (2017). Chemical composition and biological activities of lemon (Citrus limon) leaf essential oil. Nutr Food Sci Res. 4(4): 15-24.

Hong, X.X., J.G. Luo, C. Guo, and L. Y. Kong (2012). New steroidal saponins from the bulbs of *Lilium brownii* var. *viridulum.* Carbohydr. Res. 361: 19-26.

Jacobson, E., A. Dame, J. Pyrek, and M. Jacobson, (1995). Evaluating the role of niacin in human carcinogenesis. Biochimie. 77(5): 394-398.

Javed, A., M. Usman, S.M. Haider, B. Zafar, and K. Iftikhar (2019). Potential of Indigenous Plants for Skin Healing and Care. Am. Sci. Res. J. Eng., Technol., Sci. 51(1): 192-211.

Kalita, B.C., A.K. Das, D.D. Gupta, P.K. Hui, B. Gogoi, and H. Tag (2018). GC-MS analysis of phytocomponents in the methanolic extract of *Gynocardia odorata* R. Br.-A poisonous plant from Arunachal Himalayan Region. J Pharmacogn Phytochem. 7(1): 2458-2463.

Khatua, S., A. Pandey, and S.J. Biswas (2016). Phytochemical evaluation and antimicrobial properties of *Trichosanthes dioica* root extract. J Pharmacogn Phytochem. 5(5): 410-413.

Kogiannou, D.A., N. Kalogeropoulos, P. Kefalas, M.G. Polissiou, and A.C. Kaliora (2013). Herbal infusions; their phenolic profile, antioxidant and anti-inflammatory effects in HT29 and PC3 cells. Food Chem Toxicol. 61: 152-159.

Kumar, V., and J. Van Staden (2016). A review of *Swertia chirayita* (Gentianaceae) as a traditional medicinal plant. Front. Pharmacol. 6: 308.

Maini, P., J. Murray, and G. Oster (1985). A mechanical model for biological pattern formation: a nonlinear bifurcation analysis, Ordinary and Partial Differential Equations. Springer. 252-269 p.

Majnooni, M.B., B. Mohammadi, R. Jalili, A. Babaei, and G. Bahrami (2016). Determination of fatty acids by high-performance liquid chromatography and fluorescence detection using precolumn derivatization with 9-fluorenylmethyl chloroformate. J Liq Chromatogr Relat Technol. 39(19-20): 877-881.

Meena, R. (2012). Bio-efficacy of plant extracts for management of purple blotch disease of onion (*Allium cepa*). Indian Phytopathol. 65(3): 253-257.

Mishra, R., K. Srivastava, and R. Gupta (2008). Evaluation of botenical extracts in management of Stemphylium blight of onion (*Allium cepa* L.). Int. J. Veg. Sci. 35: 165-168.

Muhammad, H., M. Gomes-Carneiro, K. Poça, A. De-Oliveira, A. Afzan, S. Sulaiman, Z. Ismail, and F. Paumgartten (2011). Evaluation of the genotoxicity of Orthosiphon stamineus aqueous extract. J. Ethnopharmacol. 133(2): 647-653.

Nayak, D., S. Ashe, P.R. Rauta, and B. Nayak (2017). Assessment of antioxidant, antimicrobial and anti-osteosarcoma potential of four traditionally used Indian medicinal plants. J. Appl. Biomed.15(2): 119-132.

Omoregie, G.O., O. Ovuakporie-Uvo, and M. Idu (2018). Phyto-composition and antimicrobial activities of the ethanol seed extracts of Buchholzia coriacea Afr J Pharm Pharmacol. 7(2): 53-58.

Pandian, R.S., and A.T. Noora (2019). GC-MS Analysis of Phytochemical Compounds Present in the Leaves of *Citrus medica.* L. Research J. Pharm. and Tech.12(4): 1823-1826.

Pareek, S., N.A. Sagar, S. Sharma, S., and V. Kumar (2017). Onion (Allium cepa L.). Fruit and vegetable phytochemicals: Chemistry and human health, 2nd ed.; Yahia, EM, Ed, 1145-1161p.

Priya, R., A. Sataraddi, and S. Darshan (2015). Efficacy of non-systemic and systemic fungicides against purple blotch of onion (*Allium cepa* L.) caused by *Alternaria porri* (Ellis) Cif. Int J Recent Sci Res. 6(9): 6519-6521.

Pu, H., X. Li, Q. Du, H. Cui, and Y. Xu (2017). Research progress in the application of Chinese herbal medicines in aquaculture: A Review. Eng. 3(5): 731-737.

Rafiq M, Javaid A**,** Shoaib A (2017). Possible antifungal and antibacterial constituents in inflorescence extract of *Carthamus oxycantha. Mycopath* 15(2): 89-95.

Riaz, M., M. Zahid, M.A. Sherkheli, M. Altaf, S. Manzoor, N. Abbasi, R. Rashid, M.Q. Khan, and S. Andleeb (2019). Measurements of metal contamination, antimicrobial and antioxidant activities of five medicinal herbs from Azad Kashmir. Bangladesh pharm j. 22(1): 7-12.

Senthil, J., M. Rameashkannan, and P. Mani (2016). Phytochemical profiling of ethanolic leaves extract of Ipomoea sepiaria (Koenig Ex. Roxb). Int J Innov Res Sci Eng Technol. 5(3): 3140-3147.

Siddiqua, A., M. Zahra, K. Begum, and M. Jamil (2018). The traditional uses, phytochemistry and pharmacological properties of *Cassia fistula*. J Pharm Pharmacol Res. *2*(1): 015-023.

Thi, H.T., B.A. Le, H.N.T. Le, K. Okitsu, K. Imamura, N. Takenaka, B. Van Luu, and Y. Maeda (2018). Screening of fatty acids, saccharides, and phytochemicals in *Jatropha curcas* seed kernel as their trimethylsilyl derivatives using gas chromatography/mass spectrometry. J Chromatogr B. 1102: 66-73.

Tshabalala, T., A. Ndhlala, B. Ncube, H. Abdelgadir, and J. Van Staden (2019). Potential substitution of the root with the leaf in the use of Moringa oleifera for antimicrobial, antidiabetic and antioxidant properties. S. Afr. J. Bot. In Press.

Valsalam, S., A. Paul, M.V. Arasu, N.A. Al-Dhabi, A.K.M. Ghilan, K. Kaviyarasu, B. Ravindran, S.W. Chang, and S. Arokiyaraj (2018). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J. Photochem. Photobiol. B. 191: 65-74.

Vijayakumar, A.S., M. Jeyaraj, M. Selvakumar, and E. Abirami (2019). Pharmacological activity of silver nanoparticles ethanolic extract from *Justicia Gendarussa* (Burm) F Plant Leaves. Res. J. Pharm., Biol. Chem. Sci. 5(2):462-475

Vincent, J., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 159(4051): 850p.

Wang, Y., X. Guo, J. Xie, Z. Hou, and Y. Li (2015). A GC-MS based metabonomics study of rheumatoid arthritis and the interventional effects of the simiaowan in rats. Molecules. 20(12): 21364-21372.

Yasin, H., M. Zahoor, Z. Yousaf, A. Aftab, N. Saleh, N. Riaz, and B. Shamsheer (2019). Ethnopharmacological exploration of medicinal mushroom from Pakistan. Phytomedicine.54: 43-55.