ANTIBACTERIAL AND ANTIOXIDANT CHARACTERISTICS OF *CANNABIS SATIVA*: A MEDICINAL HERB FROM GILGIT-BALTISTAN

Rida, R. Dilshad and R. Batool*

Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, 54590, Lahore
*Corresponding author E-mail: rida.mmg@pu.edu.pk

ABSTRACT: Medicinal plants are being used from prehistoric times for the curative purposes. In present study, local plants of Gilgit-Baltistan i.e., *Cannabis sativa, Arteimisia sieversiana* and *Delphinium brunonianum* were investigated for their antibacterial and antioxidant potential. Ethyl acetate extracts of these selected plants revealed the presence of different phytochemicals in all extracts. Maximum antibacterial activity against *Bacillus* (KC 881030) and *Pseudomonas* (KC 881031) strains was shown by *C. sativa* (14 mm). Its minimum inhibition concentration (MIC) was found to be 1.56 mg/ml against test strain of *Bacillus*. Free radical scavenging ability of *C. sativa* plant extract was highest (68.9%) as compared to rest of the tested extracts. Antimitotic assay showed considerable antimitotic potential (20% mitotic index) of *C. sativa*. Thin Layer Chromatography revealed 11 different spots that were further partially purified to determine their antibacterial activity. Significant zone of inhibition were exhibited by two spots i.e. 17 mm and 18 mm. In future, bioactive components of these selected plants can be used in pharmaceutical sector as antioxidant and antimitotic agents.

Keywords: Antibacterial Activity; Antimitotic Activity; *Cannabis sativa*; Phytochemical Analysis; Thin Layer Chromatography.

(Received 20.02.2020 Accepted 11.03.2020)

INTRODUCTION

Early mankind depended on plants and animals for their food, health and shelter (Larsen 2018). Use of plants for medicinal purposes enhanced with the rise in bacterial resistance against synthetic drugs. This resistance was a consequence of misuse of antibiotics in developing countries (Kumar *et al*. 2020; Saleem 2019). With these emerging problems, traditional medicine is considered as ultimate health care remedy (Karunamoorthi *et al*. 2013). Hence, despite of advanced synthetic drugs, about 80% of people rely on plants for medication these days due to the remedial properties governed by them (Basha and Sudarshanam 2011).

Research on plant species from world reported around 258,650 species of higher plants. Among them more than 10% have been utilized as remedy for unhealthy inhabitants. About six thousand species of higher plants are found in Pakistan and out of them up to seven hundred species are known to be used as herbal medicines (Shinwari 2010). Pakistan is blessed with different climatic zone that confers diversity of the medicinal plants (Akbar 2017). Medicinal plants have been categorized into two main groups. First group includes such plants that are being used by physicians to relieve the pain whereas plants of second group are utilized in pharmaceutical industries for their bioactive compounds to manufacture synthetic drugs (Lokendrajit *et al*. 2012).

In Pakistan, local physicians (hakims) use herbs to treat illness in health care centers. Herbs play central role in preparation of homeopathic as well as allopathic medicines (Nagori *et al*. 2011). Now a days, herbal medicines become a separate industry and preferentially used by large population (Pandey *et al*. 2011). Investigation suggested that plant extracts are main ingredients of allopathic drugs and are responsible for antimicrobial, antiviral and antitumor activity (Chandra *et al*. 2017).

In research field, search for the bioactive compounds of plants have been started all over the world (Dilshad *et al*. 2018). These bioactive compounds are being implicated in pharmaceuticals, uncooked and processed food preservation, natural remedies and unconventional advanced medicines (Morin-Crini *et al*. 2019).

This study is based on preliminary analysis of medicinal plants collected from Gilgit Baltistan region of Pakistan because this area is blessed with around 3000 plant species of which 124 are identified as medicinal plants (Akhtar *et al*. 2016). Gilgit-Baltistan is mountainous area with outstanding plant diversity (Salim *et al*. 2019). Herbal medication is common in different areas of Gilgit-Baltistan and it was found that about 80% of population depends on plants for their food and medication. In allopathic drugs, medicinal plant ingredients are being used to cure illnesses effectively (Akhtar *et al*. 2016).

Cannabis sativa L. was first identified in central Asia and has been used in traditional medicines. Important phytochemicals have been identified in *C. sativa* i.e., Terpenes, phenolic compounds, and cannabinoids that showed effective bioactivities on human body and are responsible for therapeutic effect (Khan *et al*. 2010, Andre *et al*. 2016). Cannabinoids have been reported to have antibacterial potential against gram positive bacteria and yeast (Khan *et al*. 2010, Ali *et al*. 2012). In Gilgit Baltistan, *C. sativa* commonly called as Thounch which is traditionally used as a tonic, analgesic and stomachic. In winter season, baked seeds of plant are mixed with dry fruits as nuts. Oil of seeds is also used for the treatment of sore throat (Kumar *et al*. 2017).

Mentha longifolia L. which is commonly called as wild mint is cultivate widely in Mediterranean regions, Australia and Europe. M. longifolia is extensively used in various industries such as food, tobacco and pharmaceutical industry as well as use in cosmetics. It has medicinal importance in health care practices and traditionally used to cure digestive disorder and headache. Its antimicrobial potential has been identified and can be used as carminative, antispasmodic and stimulant. It has also been reported to cure throat and mouth irritation traditionally, with the leaves (Al-Bayati, 2009; Mikaili et al., 2013).

Artemisia sieversiana is one of the common specie of Artemisia which has a medicinal potential. In this specie antiviral, antioxidant and antimutagenic potential has been reported. Their flowering tops in powdered form showed efficacy for urinary problems and inflammation. Variety of secondary metabolites and aroma chemicals such a caumarin derivatives, acetylenes and flavonoids have been identified (Mangathayaru et al., 2007). Chemical analysis of essential oil revealed that azulenes are present in *A. sieversiana* which confers antifungal, antimicrobial and anthelminthic potential (Abad et al., 2012). In Gilgit Baltistan it is known khakamus.

Objective of the current study was to check the antibacterial and antioxidant activity of selected medicinal plants from the Gilgit Baltistan.

MATERIALS AND METHODS

Sampling: To collect information about medical and traditional uses of indigenous plants from northern areas of Pakistan, a survey was conducted. Plant species which were recommended by the natives for medicinal purpose were collected from district Gilgit, Gilgit-Baltistan province, Pakistan. Three different plant species were selected which were commonly used to cure several diseases i.e., *A. sieversiana* (Khakamus), *C. sativa* (Thounch) and *D. brunonianum* (Makhoti). Leaves, stem and flower of these plants were collected for the study.

Preparation of plant extracts: Local plants were collected and washed properly to remove dirt. Plants were dried at room temperature in the dark and crushed to powdered form. Powder was stored in a glass jar for future use at room temperature. In a 250ml flask, 10 gram of each plant powder was dissolved in 100ml Ethyl acetate and mixed gently. The mixture was left at room temperature for 24 hours and plant extract was filtered by using Whatman filter paper.

Phytochemical analysis of selected medicinal plant extracts: In present study, selected plants were checked for the presences of different phytochemical components in them, such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, phlobatanins, saponins, tannins, terpenoids, steroids, oxalate and proteins (Yadav and Agarwala 2011, Kavit *et al*. 2013, Ugochukwu *et al*. 2013, Pradeep *et al*. 2014)

Determination of Antibacterial potential of plant extract: Agar well diffusion method is widely used to determine whether a plant exhibit antimicrobial activity or not. Method of Irshad *et al*., was used for this purpose (Irshad *et al*. 2012). *Bacillus* (KC 881030) and *Pseudomonas* (KC 881031) strains were used as test strains for this assay. Ampicillin (10 µg/ml) was used as a standard while ethyl acetate was used as a negative control. The zones of inhibition shown by plant extracts against *Bacillus* and *Pseudomonas* strains were measured in mm (millimeter).

Minimum Inhibitory Concentration (Klančnik *et al.* 2010): Minimum inhibitory concentration (MIC) assessment is a quantitative assay to determine the minimum concentration of extract that inhibits the bacterial growth. Tetrazolium salt was used as an indicator of bacterial growth. To the 24 hours broth culture, tetrazolium salt was added. After incubation, change in color was observed. The wells in which extract concentration could not inhibit the growth of bacteria cells showed change in color due to reaction with tetrazolium salt. While, no color change showed the absence of vegetative cells in the wells due to antibacterial activity of extract.

Antioxidant assay: The ability of plant extracts to nullify the reactive oxygen species was determined by using the method of Ahmad *et al*., (2015). 2, 2-diphenyl-2-picryl hydrazyl (DPPH) was used as a free radical that changes its color from purple to yellow whenever it comes in contact with an antioxidant. The percentage of antioxidant activity of these extract were determined by using formula.

$$\%Antioxidant\ activity = \frac{absorbanceof\ control - absorbance\ of\ sample}{absorbance\ of\ control}$$

Antimitotic activity of selected plant extract: Antimitotic activity of selected medicinal plant was evaluated by using the method of Fiskesjo with modification (Fiskesjö, 1988). Roots of *Allium cepa* were used to determine the effect of plant extracts on mitotic activity. Plant extracts were used in 4:1 concentration (plant extract: water). The mitotic index was identified by counting the number of dividing cells and non-dividing cells and putting them in following formula.

$$Mitotioc\ index = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

Thin layer chromatography: Thin layer chromatography is a chromatographic technique that is specially designed to separate volatile compounds from crude extracts. To observe sugars, present in sample the method of Zschocke was used (Zschocke *et al*. 2000). n-hexane and ethyl acetate were used in 2:1 as a mobile phase.

Extracting components from TLC plates: Separates bands of plate were marked and scratched the compounds in properly labeled respective tubes. Ethyl acetate was added to each extracted compound and left in dark for 24 hours. The extract was filtered with a Whattman filter paper and filtrate was allowed to evaporate. These evaporated filtrates were then used to check their antibacterial potential.

Antibacterial assay: Disc diffusion method was applied to determine the antibacterial activity of these spots against *Pseudomonas* and *Bacillus* strains. Nutrient gar plate was inoculated by spreading a volume of respective microbial inoculum with the help of cotton swab. Discs were made from Whatman filter paper and were autoclaved. These discs were put in each of the 11 partially purified compounds and left for 24 hours. Later on, these discs were air dried and placed on plate with negative control of ethyl acetate and positive control of antibiotic disc i.e. Ampicillin (10µg/ml). Plates were incubated for 24 hours at 37◦C. After incubation, zones of inhibition were observed and measured against each component.

RESULTS AND DISCUSSION

Medicinal plants are widely used in health care practices. Medicinal properties of plant are due to their phytochemicals which are produced as secondary metabolites. These metabolites are involved in defense mechanism of plants and can help to combat with various diseases (Sharma *et al*. 2012). These compounds can act as antimicrobial agents and are responsible for therapeutic activity of medicinal plants (Pandey *et al*. 2011).

The selected medicinal plants i.e. *C. sativa*, *A. seversiana*, and *D. brunonianum* were confirmed to possess phytochemical constituents. Oxalate, saponins, terpenoid, alkaloids, flavonoids, tannins and carbohydrates are the compounds that have been identified in all of the plant extracts. Steroids, cardiac glycoside and proteins were found in only *D. brunonianum* whereas phlobatanins was found only in *Artemisia scoparia*. According to Harborne 1998, most of the plants have alkaloids, flavonoids, terpenoid, phlobatanins and tannins (Harborne 1998, Dilshad *et al*. 2018). In *C. sativa*, phytochemicals such as terpenes, phenolic compounds, cannabinoids have been identified (Andre *et al*. 2016).

Antibacterial activity of these plant extracts was ascertained against *Pseudomonas* (KC 881031) and *Bacillus* (KC 881030) strains. All of these extracts showed antibacterial activity in the range of 5-14 mm (Table 1, Figure 1). *C. sativa* showed maximum inhibition potential and had more capacity to inhibit gram positive bacteria (14 mm) than gram negative bacteria (11 mm). The reason behind this change is the difference of cell wall composition. Gram negative bacteria are surrounded by extra outer membrane which acts as a barrier to the antibacterial components thus, it is hard to cross outer membrane of gram negative bacteria (Masi *et al*. 2017). Bacterial inhibition also depends on access of compound to the bacterial cells on agar medium via penetration. If compounds have difficulty in diffusion on agar it may resulted in lesser zone of inhibition which does not mean that extract has low antibacterial potential but showing the pseudo negative results (Klančnik *et al*. 2010).

Findings of current study depicted that *C. sativa* show maximum zone. This activity of *Cannabis* is by the cannabinoid because cannabinoids have been reported to have antibacterial potential against gram positive bacteria and yeast (Ali *et al*. 2012). Minimum inhibitory concentration (MIC) assay was performed by broth micro dilution method which is preferentially used for quantitative analysis of antibacterial potential. The well in which no color change was observed was the minimum inhibitory concentration of that extract. Starting from 100mg/ml the extract concentration was diluted 50% subsequently. Thus, *A. sieversiana* showed MIC 50 mg/ml against *Bacillus* only and *C. sativa* presented MIC (1.56 mg/ml) against *Bacillus* whereas (50 mg/ml) against *Pseudomonas*. *D. brunonianum* showed inhibition only against gram negative bacteria till 2nd well (50 mg/ml). These results are summarized in Table 1 and Figure 2.

C. sativa showed significant antibacterial activity against *Bacillus*. Cannabinoids have been reported in *C. sativa* which are responsible for antibacterial potential. These results can be attributed to the fact that *C. sativa* has potential to inhibit a wide range of bacteria and fungi (Ali *et al*. 2012).

Presence of antioxidant potential was shown by all extracts but *C. sativa* had high potential of scavenging free radicals (68.9%) as compared to *A. sieversiana* (47.3%) and *D. brunonianum* (49.3%) as mentioned in Table 1. The antioxidant activity of all extracts was due to the presence of flavonoid as these phenolic compounds are effective antioxidants (Chew *et al*. 2011, Chen *et al*. 2012). *C. sativa* have greater antioxidant activity because it has high concentration of flavonoids whereas the minimum activity of *A. sieversiana* is because of lesser concentration of flavonoid which was also confirmed during phytochemical analysis.

During development process in animals and plants, the number of cells increases rapidly by mitosis. Mitosis can be disturbed by certain agents at any point of cell cycle, such type of agents are called as antimitotic agents (Dilshad *et al*. 2018). *Allium cepa* was used to test this activity because the chromosomal aberrations that are produced by any agent on plant chromosome are similar to the aberration shown by mammalian cells (Auti *et al*. 2010, Thenmozhi *et al*. 2011). The selected plant extract which showed maximum zone of inhibition i.e. *C. sativa* was used to check its antimitotic potential. Roots of *Allium cepa* were treated with plant extract, blank (solvent), and negative control (simple tap water) in respective tubes. In all of them, minimum mitotic index was shown by plant extract i.e. 20% which revealed that it had maximum antimitotic activity (Table 1, Figure 3). This activity of plant extract is because of phytochemical constituents. Physiological functions of cells such as mitosis have been altered by the action of glycosides, alkaloid or steroidal alcohol, polyphenols, and flavonoids which can stop proliferation of cancer cells (Thenmozhi *et al*. 2011).

C. sativa (Thounch) was the one that showed maximum antibacterial, antioxidant and antimitotic potential. So, this plant was selected to determine the components that were responsible for its antibacterial potential. Eleven bands of various bioactive components, in this extract, were separated by TLC (Figure 4). Brown spot was observed when developed in hexane and ethyl acetate solvent system (1:2) and sprayed with sulphuric acid. The brown spot indicated the presence of sugar in extract as reported by Chavan and Amarowicz, (2013).

All of these components were checked for their antibacterial activity. Spot 4 to 9 were positive for antibacterial activity with R_f value ranging from 0.47 to 0.84. The zone of inhibition of spots were in such a manner that suggested the synergic effect of components as shown in Figure 5. Synergistic effect of different phytochemicals is also reported in case of *Camellia sinensis* and *Juglans regia* (Farooqui *et al.* 2015).

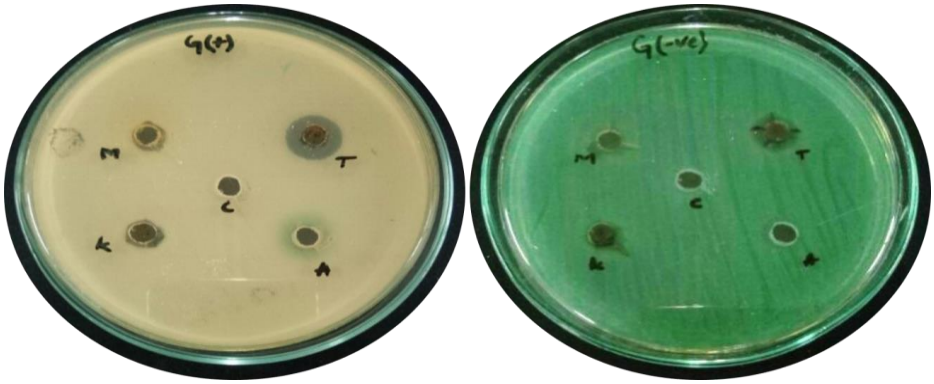

Figure 1. Antibacterial activity of *Artemisia* (K), *Cannabis* (T), and *Delphinium* (M), with ampicillin (A) as a standard and Ethyl acetate (C) as a control. Left: Against B*acillus*. Right: Against *Pseudomonas.*

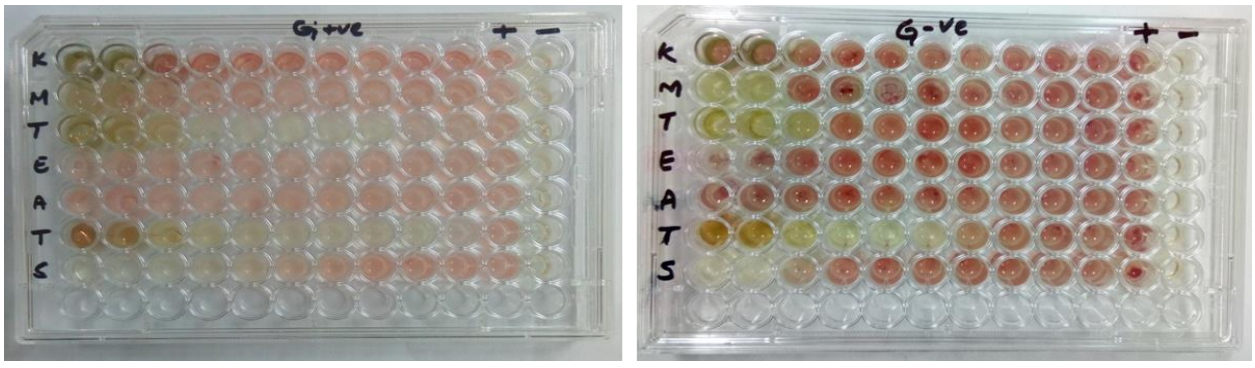

Figure 2. MIC assay of plant extracts labelled as *Artemisia* (K), *Cannabis* (T), and *Delphinium* (M) while E being the ethyl acetate control. A (Ampicillin), T (tetracyclin) and S (Streptomycin) were used as standards. Left: Activity against gram positive strain Right: Activity against gram negative strain.

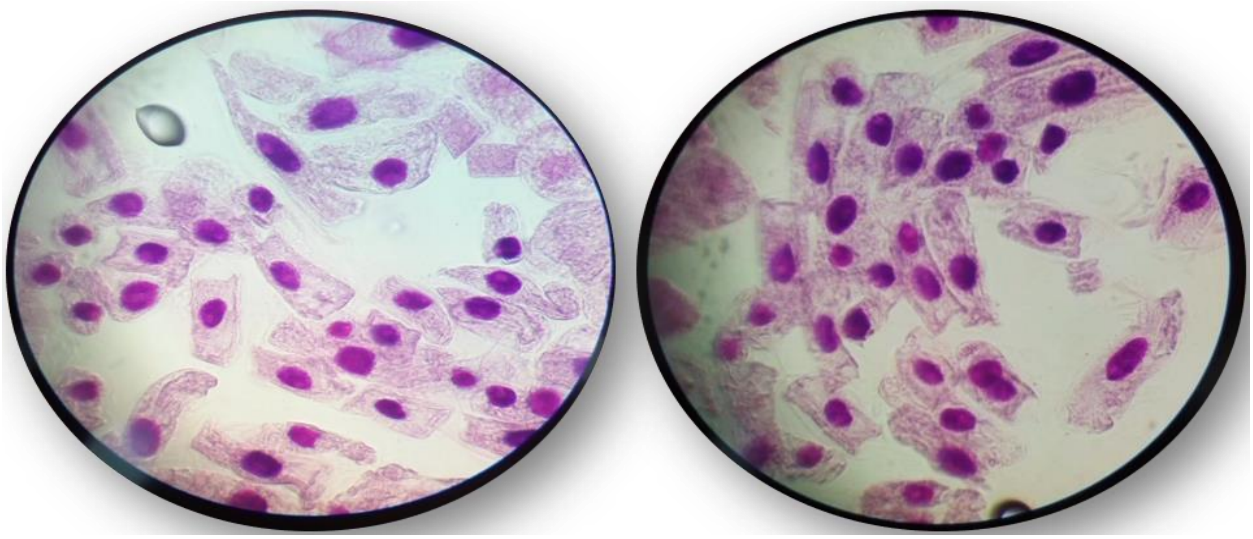

Figure 3. Antimitotic assay; Left: Root cells of *Allium cepa* grown with extract of *C. sativa,* Right: Untreated root cells (control).

Figure 4: Thin Layer Chromatography plate of *C. sativa* extract.

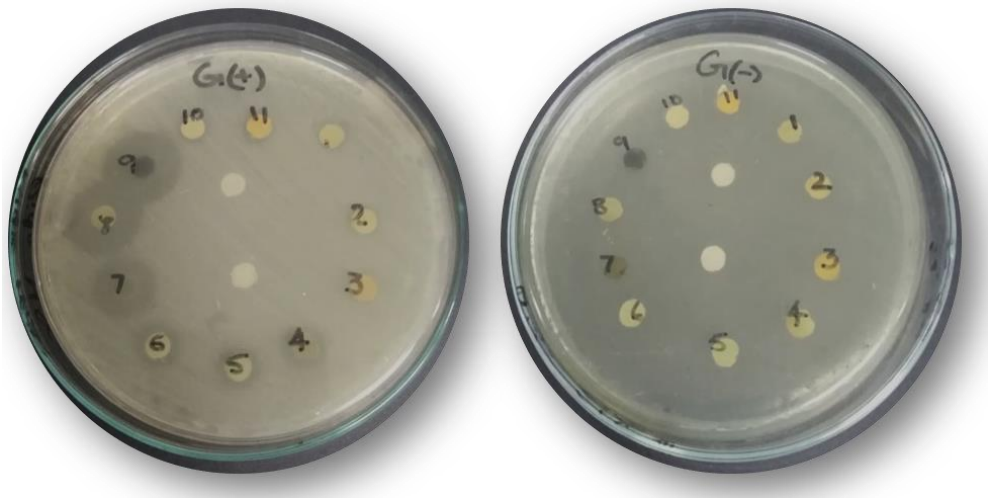

Table 1. Phytochemical analysis, antibacterial and antioxidant activity of extracts of selected plants.

Plant Extract Used	Phytochemicals											Antibacterial Activity (mm)		Minimum Inhibitory Concentration (mg/ml)	Antioxidant Activity (%)	Mitotic Index (%)
	Alkaloids	Carbohydrate	Cardiac glycoside	Flavonoids	Phlobatanins	Saponins	Tannins	Terpenoid	Steroids	Oxalate	Proteins	*Bacillus*	*Pseudomonas*			
A	+	+	-	+	+	+	+	+	-	+	-	8±0.47	7±0.47	1.56	47.3±0.18	20±0.47
C	+	+	+	+	+	+	+	+	-	+	-	14±0.47	11±0.47	50	68.9±0.35	48±0.47
D	+	+	-	+	-	+	+	+	+	+	+	6±0.47	5±0.47	50	49.3±0.35	52.5±0.23

A= *A. sieversiana,* C= *C. sativa* and D= *D. brunonianum*, Mean of three replicates, ± Standard error of mean

Conclusion: Plants that are traditionally used to cure illnesses are studied to evaluate the biological mechanisms to combat with microbes. It was found that *C. sativa, A. sieversiana,* and *D. brunonianum* have antimicrobial, antioxidant and antimitotic potential. Current study revealed that local plants are pharmacologically important and their phytochemicals can be isolated from the crude extracts for the synthesis of new drugs. Hence, these selected plants can be used to produce alternative drugs against conventional antibiotics to fight against drug resistant microbes and their pharmaceutical practice can reduce the economic burden.

REFERENCES

Abad, M. J., L. M. Bedoya, L. Apaza and P. Bermejo (2012). The Artemisia L. genus: a review of bioactive essential oils. Molecules. 17(3): 2542-2566.

Akbar, K. (2017). Potential impacts of climate change on plant diversity of hilly areas of Azad Kashmir and their mitigation: a review. J. Mt. Area Res. 2: 37-44.

Akhtar, N., S. Akhtar, S. Kazim and T. Khan (2016). Ethnomedicinal study of important medicinal plants used for gynecological issues among rural women folk in district Gilgit, Pakistan. Nat. Sci. 14(9): 30-34.

Al-Bayati, F. A. (2009). Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. Ann Clin Microbiol Antimicrob. 8(1): 20.

Ali, E.M., A.Z. Almagboul, S.M. Khogali and U.M. Gergeir (2012). Antimicrobial activity of *Cannabis sativa* L. Chin. Med. 3(01): 61-64.

Andre, C. M., J.F. Hausman and G. Guerriero (2016). *Cannabis sativa*: the plant of the thousand and one molecules. Front. Plant. Sci. 7: 1-19. doi: 10.3389/fpls.2016.00019.

Auti, S., R. Pagare, D. Ahire and V. Sawale (2010). Cytogenetical studies on the effect of omnacortil on root tip cells of Allium cepa L. J. Cell. Tissue. Res. 10(3): 2331-2335.

Basha, S. K. and G. Sudarshanam (2011). Multiple herbal therapy: Antimicrobial activity of wound healing paste (Pasuru) used by sugali tribes of Yerramalais of Kurnool district, Andhra Pradesh, India. Int. J. Pharm. Tech. Res. 3(3): 1238-1241.

Chandra, H., P. Bishnoi, A. Yadav, B. Patni, A. P. Mishra and A. R. Nautiyal (2017). Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—a review. Plants. 6(2): 1-16.

Chen, T., J. He, J. Zhang, X. Li, H. Zhang, J. Hao and L. Li (2012). The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of Cannabis sativa L.). Food. Chem. 134(2): 1030-1037.

Chew, Y. L., E.W.L. Chan, P.L. Tan, Y.Y. Lim, J. Stanslas and J.K. Goh (2011). Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC. Complement. Altern. Med. 11(1): 12. doi: 10.1186/1472-6882-11-12.

Dilshad, R., R. Batool and N. Jamil (2018). Phytochemical screening and antibacterial potential of *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus*. Pak. J. Pharm. Sci. 31(2): 499-507.

Farooqui, A., A. Khan, I. Borghetto, S.U. Kazmi, S. Rubino and B. Paglietti (2015). Synergistic antimicrobial activity of *Camellia sinensis* and *Juglans regia* against multidrug-resistant bacteria. PloS one, 10(2): e0118431. https://doi.org/10.1371/journal.pone.0118431

Harborne, A. (1998). Phytochemical methods a guide to modern techniques of plant analysis, springer science & business media.

Irshad, S., M. Mahmood and F. Perveen (2012). In vitro antibacterial activities of three medicinal plants using agar well diffusion method. Res. J. Biol. 2(1): 1-8.

Karunamoorthi, K., K. Jegajeevanram, J. Vijayalakshmi and E. Mengistie (2013). Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. J. Evid. Based. Complement. Alternat. Med. 18(1): 67-74.

Kavit, M., B. Patel and B. Jain (2013). Phytochemical analysis of leaf extract of *Phyllanthus fraternus*. Res. J. Recent Sci. ISSN 2: 12-15.

Khan, R.U., F. Durrani, N. Chand and H. Anwar (2010). Influence of feed supplementation with Cannabis sativa on quality of broilers carcass. Pak. Vet. J. 30(1): 34-38.

Klančnik, A., S. Piskernik, B. Jeršek and S. S. Možina (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J. Microbiol. Methods. 81(2): 121-126.

Kumar, P., J. H. Lee, H. Beyenal and J. Lee (2020). Fatty Acids as Antibiofilm and Antivirulence Agents. Trends Microbiol. (in press).

Kumar, S., B.S. Singh and R. Singh (2017). Ethnomedicinal plants uses to cure different human diseases by rural and tribal peoples of Hathras district of Uttar Pradesh. J. Pharmacogn. Phytochem. 6(2): 346-348.

Larsen, C.S. (2018). Bioarchaeology. Int. Encycl. Biol. Anthropol.: 1-14.

Lokendrajit, N., S. I. Devi, N. Swapana and C. Singh (2012). Ethno-medicinal plants of Manipur used in the treatment of blood pressure. NeBio. 3(1): 39-41.

Mangathayaru, K., K. Sravan, A. P. K. Reddy, and R. M. Kumar (2007). PHCOG MAG.: Research Article In vitro antioxidant studies of the aerial parts of *Origanum majoram* Linn and *Artemesia sieversiana* Ehrh. Pharmacog Mag. 3(10): 90.

Masi, M., M. Réfregiers, K.M. Pos and J.M. Pagès (2017). Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. Nat. Microbiol. 2(3): 1-7.

Mikaili, P., S. Mojaverrostami, M. Moloudizargari and S. Aghajanshakeri (2013). Pharmacological and therapeutic effects of Mentha Longifolia L. and its main constituent, menthol. Anc Sci Life. 33(2): 131.

Morin-Crini, N., E. Lichtfouse, G. Torri and G. Crini (2019). Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry. Environ. Chem. Letters: 17: 1667–1692.

Nagori, K., M. Sharma, A. Agrawal, A. K. Agarwal, A. Sharma, H. Verma and D. Tripathi (2011). General awareness on allopathic, ayurvedic and homeopathic system of medicine in Chhattisgarh, India. Int. J. Pharm. Pharm. Sci. 3(4): 159-162.

Pandey, M., M. Debnath, S. Gupta and S. K. Chikara (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. J. Pharmacognosy. Phytother. 3(1): 113-117.

Pradeep, A., M. Dinesh, A. Govindaraj, D. Vinothkumar and N. Ramesh Babu (2014). Phytochemical analysis of some important medicinal plants. Int. J. Biol. Pharm. Res. 5(1): 48-50.

Saleem, M. (2019). Antibiotics overuse and bacterial resistance. Ann. Microbiol. Res. 3(1): 93-99.

Salim, M.A., S. Ranjitkar, R. Hart, T. Khan, S. Ali, C. Kiran, A. Parveen, Z. Batool, S. Bano and J. Xu (2019). Regional trade of medicinal plants has facilitated the retention of traditional knowledge: case study in Gilgit-Baltistan Pakistan. J. Ethnobiol. Ethnomed. 15(1): 6.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 2012: 1-26.

Shinwari, Z.K. (2010). Medicinal plants research in Pakistan. J. Med. Plants. Res. 4(3): 161-176.

Thenmozhi, A., A. Nagalakshmi and U. Mahadeva Rao (2011). Study of cytotoxic and antimitotic activities of Solanum nigrum by using *Allium cepa* root tip assay and cancer chemo preventive activity using MCF-7-human mammary gland breast adenocarcinoma cell lines. Int. J. Sci. Technol. 1(2): 26-48.

Ugochukwu, S.C., A. Uche and O. Ifeanyi (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian J. Plant. Sci. Res. 3(3): 10-13.

Xu, Q., X. Wen and X. Deng (2004). A simple protocol for isolating genomic DNA from chestnut rose (Rosa roxburghii Tratt) for RFLP and PCR analyses. Plant. Mol. Biol. Rep. 22(3): 301-302.

Yadav, R. and M. Agarwala (2011). Phytochemical analysis of some medicinal plants. J. Phytol. 3(12): 10-14.

Zschocke, S., T. Rabe, J. Taylor, A. Jäger and J.V. Staden (2000). Plant part substitution–a way to conserve endangered medicinal plants? J. Ethnopharmacol. 71(1-2): 281-292.