In-Vitro Antibacterial Activities of n-Hexane, Methanolic and Ethanolic Extracts of *Psidium guajava, Helianthus annuus* and *Mentha longifolia*

Tahir Hameed*[1,3], Muhammad Nisar[1], Izhar Ahmad[3], Khaista Rahman[3] Rukhsana Ghaffar[2], Shariat Ullah[1], Muhammad Ikram[4], Ali Bahadur[5]

[1]Department of Botany, University of Malakand, Chakdara, Dir (L), Pakistan

[2]Department of Pharmacy, University of Malakand, Chakdara, Dir (L), Pakistan

[3]Department of Botany, Islamia College, Peshawar, Pakistan

[4]Department of Botany, Hazara University Mansehra, Pakistan

[5]State Key Laboratory of Cryospheric Science, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

***Corresponding Author Email**: Tahir Hameed

Abstract

Antibacterial agents obtained from traditional medicinal plants are remarkable source for treating various diseases caused by micro-organisms. The three medicinal plants widely used in folklore remedies, showed high activity. Ethanol, Methanol, and n-hexane extracts of *Psidium guajava* L. leaves, *Helianthus annus* L. leaves and aerial parts of *Mentha longifolia* L. were screened for potential antibacterial activity against 5 bacterial strains, two Gram positive bacteria (*Bacillus cereus and Bacillus subtilis*) and three Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) using agar well diffusion assay. Most of the extracts showed relatively high antibacterial activity against all the tested bacteria with the diameter of inhibition zone ranging between 8 and 30.1mm.The ethanolic extract was more active than methanol and n-hexane extracts. The n-hexane extracts of most plants were inactive against *Salmonella typhi*. The most active extracts were those obtained from leaves of *psidium guajava* with inhibition zone30.1mm.The combined effect of ethanol extract of (*Psidium guajava* L / *Helianthus annus* L) on bacterial species (*B. subtilis, S. typhi)* exhibited a higher zone (31.3mm) than that of any single extract. These medicinal plants could be a source of new antibiotic compounds; further work is needed, to isolate the secondary metabolites /natural products from the crude active extract.

Keywords: *Psidium guajava*, *Helianthus annus*, *Mentha longifolia*., *Agar well diffusion assay*, *Gram positive/ Gram negative bacteria*

1. INTRODUCTION

Medicinal plants are plants that have been shown to have active biochemical constituents and to help treat illnesses in living beings. These plants have been given the name "medicinal plants" (Hameed *et al*., 2023). The medicinal plants would be the best source to obtain a variety of drugs and herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (World Health Organization, 2001). Medicinal plants represent a rich source of antimicrobial agents which are used medicinally in different countries and are a source of many effective and powerful drugs (Srivastava *et al*., 1996). Plants has used as medicine for thousands of years (Samuelsson, 2004) which are initially took in the form of crud drugs such as tincture, teas, poultices, powder, and other herbal formulation (Samuelsson, 2004; Balick and Cox, 1997). Nowadays ,it is generally accepted that certain classes of plant-based compounds such as dietary fiber, phenolic acids, flavonoids, vitamins, and antimicrobial agents and neuro-pharmacological agents play Preventive function against the prevalence of some common diseases like cancer, cardiovascular and neurodegenerative disorders (Liu, 2008; Siddhuraju & Becker, 2007 and Fan *et al*., 2007). Many of anti-microbial agents obtained from traditional medicinal plants are available for treating various diseases caused by micro-organisms. The resistance of pathogenic Bacterial strains to antibiotics is the major burning issue around the world (Rahman *et al*., 2015). According to World Health Organization (2001) has also reported that infectious and parasitic diseases account for 26.2% of the global cause of death, the large number of which occurred in developing countries, therefore, actions must be taken to reduce this problem such as controlling the misuse of antibiotics and continuing investigations aimed at the development of drugs from natural sources (Austan, 1999). *Psidium guajava* L. belongs to family Myrtaceae is widely recognized as a plant of many herbal medicines. The leaf extract of the plant was found to possess analgesic, anti-inflammatory properties, (Nundkumar and Ojewole, 2002) antimicrobial (Nair, 2007) hepatic-protective and antioxidant activities (Vyas, 2010). *Helianthus annus* L (family Asteraceae) planted in Pakistan over an area of 61,900 hectares producing 87,100 tons annually. The Leaves of *H. annus* L. have anti-microbial activity against many bacteria

(Mitscher, 1983). *Mentha longifolia* L. belongs to family Labiatae. The *Mentha longifolia* L posses various activities, such as antimicrobial and antioxidant (Mimica-Duki, *et al*., 1999) antimycotic (Abou-Jawdah *et al*., 2002), anti HIV (Amzazi *et al*., 2003) and anth-elmintic (Kozan *et al*., 2006). The present study aims to check the antibacterial activity of ethanol, methanol and n-hexane extracts of the leaves of *Psidium guajava* L, *Helianthus annus* L and aerial parts of *Mentha longifolia* L. growing in Malakand Division Khyber Pakhtunkhwa, Pakistan.

2. MATERIALS AND METHODS

2.1 Plant materials collection and identification

In the present study three medicinal plants species, *Psidium guajava, Helianthus annuus* and *Mentha longifolia* were collected from different areas of Malakand Division Khyber Pakhtunkhwa, Pakistan. The plant species were identified with the help of taxonomists, previous available literature (Nasir & Ali, 1970-1989; Ali & Nasir, 1989-1992).

2.2 Ethno botanical study

The ethnomedicinal data was collected through face-to-face interviews, group discussion, and semi-structured questionnaires. and informants were selected through snowball and free listing method (Ghorbani et al. 2011; Jan et al. 2022). All the informants were interviewed in their native language (Pashto). The interviews from the male informants were taken in the field, Hujra, or Baithaks, and the interviews from female informants were taken at homes. To document the present status of traditional knowledge the local herbalists (hakims) were also interrogated in their herbal shops

2.3 Preparation of extract

The dried undamaged plant parts; leaves and aerial part were selected and grounded into coarse powder form through electric blender. Sample (300 gm) of each plant powder was soaked in ethanol, methanol and n-hexane as a solvent and regularly shacked for (48 Hours) at 80 rpm. The extracts were filtered through Whattman filter paper and dried at 35°C under rotary vacuum. The dry extracts were stored at 4°C for further use.

2.4 Preparation of Extract and Bacterial Strain

Serial dilutions (25 mg/ml, 50 mg/ml and 100 mg/ml) of plant extract were carried out. In this study, Ciprofloxacin was used as a reference anti-biotic. Two Gram-positive (*Bacillus cereus, Bacillus subtilis*) and three gram-negative (*Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*) bacterial strains were clinical isolates obtained from the Department of Pharmacy, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan.

2.5 Growth media

Nutrient agar media are best growth media for bacteria. The Media was composed of Beef extract 3.0g, Agar 15.0g and Peptone 5.0g. One liter media were prepared by dissolving 40g of nutrient agar in 700ml of distilled water. After complete dissolution, the final volume of the media was raised to 1000ml by adding more distilled water. The media were boiled using a hot plate. The PH was adjusted to 7.0 at 25ºC, using 0.1M NaOH and 0.1M HCl. The needed media and all glassware were sterilized through autoclaving at 15psi at 121 for 20 minutes.

2.6 Antibacterial activity

The antibacterial activity of different plant crude extracts was checked through Agar well diffusion assay (Cole, 1994; Okeke et al., 2001). Briefly, the 20ml of nutrient agar was plated in Petri dishes and allowed to solidify for 30 minutes. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. Five wells (four on the periphery and one in the center) were prepared in the agar plates. A drop of molten nutrient agar was used to seal the base of each well. The wells were filled with 50µℓ of the extract concentration of 25mg/ml, 50mg/ml and 100mg/ml. The wells were filled with 50µℓ (use micropipette) of the extract concentration of 25mg/ml, 50mg/ml and 100mg/ml and allow diffusing for 45 minutes. In the central well 50µℓ of 90% n-hexane and 70% methanol was used as negative control for respective extracts. A standard disk of ciprofloxacin was used as positive controls. The antibacterial activity was also determined for combined plant extract. After holding the plates at room temperature for one hour to allow diffusion of the extract in to the agar, they are incubated for 24hours at 37˚C. After 24 hours, zone of inhibition were observed, recorded and compared with standard (Ciprofloxacin). Each sample was used in triplicate for the determination of antibacterial activity. The work was carried out in laminar flow.

2. RESULTS

The ethnomedicinal information of each medicinal plant species, including habit, local name, botanical name and utilization are investigated (Table 1). The ethanol, methanol and n-hexane extracts of *Psidium guajava* L leaves, *Helianthus annus* L. leaves and aerial parts of *Mentha longifolia* L. were screened against five species of microorganism: two Gram positive bacteria (*Bacillus cereus, Bacillus subtilis*) and three Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) (Table 2). Ethanolic extract was more active than the Methanolic and n-hexane extract. The ethanolic extracted was able to inhibit the growth of all tested organism while n-hexane extract moderately inhibit the growth of tested bacteria. The ethanolic extracts of the plants showed best antibacterial activities. The diameter of inhibition zone obtained from selected plant extracts ranges from10.0 to 30.1mm, 8.6 to 27.0mm and 10.0 to 20.2mm for ethanolic, methanolic and n-hexane extract. The highest antibacterial activity with inhibition zone (30.1mm) at concentration of 100 mg/ml were observed from the single ethanolic extract of *Psidium guajava* L leaves against *B. subtilis,* also methanolic extract of *Psidium guajava* L showed largest inhibition zone (27.0mm) against *E. coli* while (23.0mm) from n-hexane extract of *Helianthus annus* L leaves against *B.cereus.*Thelowest antibacterial activity (9.2mm) inhibition zone of leaves of *Helianthus annus* were observed against *Pseudomonas aeruginosa* at concentration of 50mg/ml andaerial parts of *Mentha longifolia* L. showed 10.0 mm inhibition zone against *Salmonella typhi.* The extracts compared with the standard antibiotic Ciprofloxacin. The antibacterial activities of combined plant extract are shown in (Table 3). The combined ethanol, methanol and n-hexane extracts of *psidium guajava,Helianthus annus* and *Mentha longifolia* were screened against *B. subtilis and S. typhi.* The diameter of inhibition zone of the combined ethanol, methanol and n-hexane extracts of the three plants ranges from 11.2 to 31.3mm. The largest inhibition zone (31.3mm) was obtained from ethanol/ethanol extract of *Psidium guajava* and *Helianthus annus*.

Table 1: Ethno botanical study of selected medicinal plants

S/No	Botanical name	Family	Local Name	Habit	Medicinal Uses
1	*Psidium guajava*	Myrtaceae	Amrod	Tree	It is used in treatment of diarrhea, dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief, cough, oral ulcers and to improve locomotors coordination and liver damage inflammation
2	*Helianthus annus* L	Asteraceae	Nwarparas	Herb	It is used on snakebites and insect bites, Anti-inflammatory, antitumor, antigen, antipyretic, reduces high fevers, cough flower tea used for lung ailments, sunflower oil used to reduce both total cholesterol and low-density lipoprotein (LDL) cholesterol and offer antioxidant properties.
3	*Mentha longifolia* L	Lamiaceae	Inaly	Herb	It is used as a treatment for colic, menstrual disorders, indigestion, flatulence, pulmonary infections and congestion, headaches, fever, coughs, colds, and urinary tract infections.

Table 1: Anti-bacterial Activity of different medicinal plants extract

Plants Name	Solvent	Con. of Extract (mg/ml)	Inhibition zone (mm) (Mean ± SD)				
			Gram positive		Gram negative		
			B. subtilis	*B. cereus*	*E. coli*	*P.aeruginosa*	*S. typhi*
Psidium guajava L	Ethanol	25.00	18.1 ± 0.4	13.2 ± 0.3	20.5 ± 0.2	15.2 ± 0.2	14.0 ± 0.1
		50.00	17.2 ± 0.1	18.2 ± 0.3	22.4 ± 0.1	20.3 ± 0.2	23.5 ± 0.4
		100.00	30.1 ± 0.1	21.3 ± 0.4	18.1 ± 0.1	22.2 ± 0.3	19.0 ± 0.1
	Methanol	25.00	16.4 ± 0.3	12.3 ± 0.2	14.2 ± 0.1	13.6 ± 0.1	10.5 ± 0.4
		50.00	18.3 ± 0.5	16.2 ± 0.1	18.5 ± 0.2	19.3 ± 0.3	13.2 ± 0.1
		100.00	27.0 ± 0.6	20.3 ± 0.4	25.3 ± 0.3	26.3 ± 0.5	18.5 ± 0.2
	n-hexane	25.00	10.0 ± 0.3	-	-	-	-
		50.00	14.3 ± 0.5	12.0 ± 0.1	12.7 ± 0.5	15.3 ± 0.2	-
		100.00	21.6 ± .8	18.2 ± 0.4	20.4 ± 0.3	20.3 ± 0.1	-
Helianthus annus L	Ethanol	25.00	-	15.2 ± 0.2	19.3 ± 0.5	14.2 ± 0.1	13.2 ± 0.2
		50.00	13.1 ± 0.3	11.4 ± 0.5	20.0 ± 0.2	16.1 ± 0.3	14.2 ± 0.2
		100.00	20.3 ± 0.2	20.5 ± 0.2	27.2 ± 0.1	20.4 ± 0.1	18.2 ± 0.3
	Methanol	25.00	16.2 ± 0.1	11.2 ± 0.3	10.4 ± 0.4	-	-
		50.00	17.3 ± 0.3	17.3 ± 0.1	15.2 ± 0.4	9.2 ± 0.1	10.3 ± 0.5
		100.00	21.0 ± 0.2	24.6 ± 0.4	22.2 ± 0.3	11.3 ± 0.4	15.3 ± 0.3
	n-hexane	25.00	10.1 ± 0.4	-	-	-	-
		50.00	15.6 ± 0.5	12.6 ± 0.5	18.4 ± 0.3	12.2 ± 0.1	-
		100.00	20.2 ± 0.4	15.0 ± 0.2	21.6 ± 0.1	18.3 ± 0.1	-

Mentha longifolia L	Ethanol	25.00	17.4 ± 0.3	14.3 ± 0.4	18.3 ± 0.2	-	-
		50.00	20.3 ± 0.2	14.2 ± 0.2	22.0 ± 0.1	10.1 ± 0.2	-
		100.00	22.2 ± 0.3	20.2 ± 0.5	26.3 ± 0.2	15.2 ± 0.2	10.0 ± 0.2
	Methanol	25.00	15.3 ± 0.3	-	15.2 ± 0.2	8.6 ± 0.2	-
		50.00	18.2 ± 0.4	13.2 ± 0.2	21.5 ± 0.5	13.6 ± 0.5	-
		100.00	23.4 ± 0.2	20.0 ± 0.4	24.4 ± 0.3	17.3 ± 0.1	12.0 ± 0.5
	n-hexane	25.00	-	-	-	-	-
		50.00	13.2 ± 0.2	-	-	11.4 ± 0.3	-
		100.00	17.1 ± 0.1	-	15.4 ± 0.5	15.1 ± 0.6	-
Ciprofloxacin (μg/ml)		25.00	20.2 ± 0.2	18.6 ± 0.1	25.2 ± 0.3	19.2 ± 0.2	17.2 ± 0.5
		50.00	25.3 ± 0.4	22.9 ± 0.2	29.9 ± 0.2	24.1 ± 0.4	22.5 ± 0.3
		100.00	31.1 ± 0.5	26.1 ± 0.4	35.8 ± 0.3	30.4 ± 0.1	26.5 ± 0.2

Table 3: Antibacterial Activity of combined plants extract on two bacterial strains

Plants Name	Combined plant extracts	Inhibition zone (mm) (Mean ± SD)	
		Gram positive	Gram negative
		B. subtilis	*S. typhi.*
Psidium guajava / *Helianthus annus*	Ethanol / Ethanol	31.3 ± 0.2	29.4 ± 0.1
	Methanol / Methanol	28.2 ± 0.2	25.3 ± 0.3
	n-hexane / n-hexane	20.2 ± 0.4	12.3 ± 0.5
Psidium guajava /*Mentha longifolia*	Ethanol / Ethanol	27.6 ± 0.3	19.5 ± 0.1
	Methanol / Methanol	22.3 ± 0.2	15.4 ± 0.2
	n-hexane / n-hexane	14.3 ± 0.2	18.4 ± 0.2
Helianthus annus /*Mentha longifolia*	Ethanol / Ethanol	22.2 ± 0.4	16.3 ± 0.5
	Methanol / Methanol	20.2 ± 0.1	24.4 ± 0.3
	n-hexane / n-hexane	18.4 ± 0.2	11.2 ± 0.5
Ciprofloxacin 100 (µg/ml)		31.1 ± 0.5	26.5 ± 0.2

4. DISCUSSION

The major traditional uses of selected medicinal plant parts were leaves, followed by fruit and seeds. Our results are supported by Dolatkhahi *et al.* (2014) and Kona and Rahman *et al.* (2016) who reported leaves as the major plant part used, followed by fruits. In the present study both single and combined extracts of medicinal plants were screened against Gram positive and Gram negative bacteria. The results showed that the plant extracts were more active against Gram positive than Gram negative bacteria. The single ethanol and methanol extract of plant showed best antibacterial activity against both gram positive and gram negative bacteria while that of n-hexane extract of all the tested plant were inactive against *S. typhi.* The ethanol leaf extract of *Psidium guajava* showed highest antibacterial activity against all the tested bacteria and high inhibition zone were observed against *Bacillus subtilis Salmonella typhi* which is in consensus with (Sanches, *et al*., 2005) that ethanol: water extracts of leaves stem bark and roots of *P. guajava* showed high activity against the gram-positive bacteria *Staphylococcus aureus and Bacillus subtilis.* The methanolic leaf extract of *Psidium guajava was active* against both gram positive and gram negative bacteria strain and this is in agreement with the report of (Abdelrahim *et al*., 2002) that methanolic extract of *Psidium guajava* was more effective in inhibiting the growth of *B. subtilis, S. aureus, E. coli* and *P. aeruginosa*. The n-hexane extract of *Psidium guajava* L. leaves was active against *Bacillus subtilis* at all concentration but completely inactive against *Salmonella typhi.* The methanolic leaf extract of *Helianthus annus* L. showed highest activity against, *Bacillus cereus* and *E. coli* (Sharma *et al*., 2009) reported that *Helianthus annuus* of methanolic extract showed activity against *Bacillus subtilis* and *Escherichia coli.* The n-hexane extract of *Helianthus annus* leaves showed good antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* but failed against *Salmonella typhi*. The ethanolic leaf extract of *Mentha longifolia* exhibited strong antibacterial activity against all the tested microorganism (Cushnie *et al*., 2003). Methanolic leaf extrac of *Mentha longifolia* L. showed good antibacterial activity against *Escherichia coli.* Similar result occurred with (Hafedh *et al*., 2010) stated that *Escherichia coli* were the most susceptible bacteria to essential oil of *Mentha longifolia* L. The n-hexane extract of *Mentha longifolia* L. leaves were active against *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* but completely failed against *Bacillus cereus* and *Salmonella typhi.* Our result is in correlation with (Khurram *et al*., 2009) previously reported that n-hexane extract of *Dodonaea*

viscosa showed high activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.* In addition the largest inhibition zones were observed from E/E combined extract of *Psidium guajava*, *Helianthus annus* against *Bacillus subtilis* and *Salmonella typhi.* Abu-Shanab *et al* (2008) reported that widest zone of inhibition was shown by combined E/E extract of *Sacropoterium spinosum and Lawsoniainerm is* against *MRSA strain 2* and *Pseudomonas aeuroginosa.* In the present research, the Ciprofloxacin antibiotic showed highest zone of inhibition against the tested bacterial strains. Our result are in line with Ahmad et al (2023), they also reported that Ciprofloxacin antibiotic reveled highest activity against the bacterial strains.

3. CONCLUSION

The aerial parts of *menthe longifolia*, leaves of *psidium guajava* and *helianthus annus* showed remarkable antibacterial activity. All the three plants contain active antibacterial compounds. It is recommended that further investigation of crude extract is needed to isolate the active antibacterial agents which may be used to formulate new and more effective antibacterial drugs of natural origin.

Conflict of Interest

The authors declared that the present study was performed in absence of any conflict of interest.

Acknowledgment

The present work is a part of BS (Botany) research project of the first author (Tahir Hameed). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Abdelrahim, S.I., A.Z. Almagboul, M. E. Omer and A. Elegami, 2002. Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive Bacteria, *Fitoterapia*, 73(7-8): 713-715.

Abou-Jawdah, Y., H. Sobh, A. Salameh, 2002. Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi, Journal of Agricultural and Food Chemistry, 50: 3208–3213.

Abu-Shanab, B.A., M. Ghaleb, A.M. Adwan, Kamel and F.B.A. Shanab, 2008. Efficacy of Aqueous and Ethanol Extracts of Some Palestinian Medicinal Plants for Potential Antibacterial Activity. The Islamic University Journal,16(2): 77-86.

Ahmad, B ., A. A. Khan., H. Maria., S, Zaman., A, Gul., A, Ali., I, Ullah., M. U. Rehman., D. Naz and A, Aziz. 2023. Antibacterial effect of Bidens bipinnata L. Extract against selected bacterial strains. Journal of Xi'an Shiyou University, Natural Science Edition, 19 (2): 1626-1638.

Ali SI, Qaiser M. 2004. Flora of Pakistan, Department of Botany, University of Karachi, Pakistan.

Amzazi, S., S. Ghoulami, Y. Bakri,A. Idrissi and S. Fkih-Tetouani, A. Benjouad, 2003. Human immunodeficiency virus type 1 inhibitory activity of *Mentha longifolia*,Therapie, 58 (6): 531–4.

Austan, D.J., K.G. Kristinsson, R.M. Anderson, 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance, Tropical Journal of Pharmaceutical Research,3(2): 225-228.

Balick M. J., P.A. Cox, 1997. Plants people and culture: the science of ethnobotany, Scientific American Library, New York NY.

Cole, M.D.,1994. Key antifungal, antibacterial and anti-insect assays-a critical review, *Biochemistry System Ecology*, 22: 837-856.

Cushnie, T.P., V.E.S. Hamilthoh and A.J.Lamb,2003. Assessment of the antimicrobial activity of selected flavonoids and consideration of discrepancies between previous reports,*Microbiology Research*, 158(4):281-291.

Dolatkhahi, M., A. Dolatkhahi and J. B. Nejad. 2014. Ethnobotanical study of medicinal plants used in Arjan – Parishan protected area in Fars Province of Iran. *Avicenna J. Phytomed.*4. 6, 402-412.

Fan, J., X. Ding, W. Gu, 2007. Radical-scavenging proanthocyanidins from sea buckthorn seed. Food Chemistry,102: 168-177.

Hafedh, H., B.A. Fethi, S. Mejdi,N. Emira and B. Amina, 2010. Effect of *Mentha longifolia* L. ssp longifolia essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. African Journal of Microbiology Research, 4 (11):1122-1127.

Hameed, T., I, Ahmad., S, Ullah, and W, Hussain. "Ethnobotanical study and conservation status of medicinal plants used by traditional healers in Toormang Valley, Northern Pakistan." Journal of Xi'an Shiyou University, Natural Science Edition, 19 (1): 1566-1594

Khurram, M.A. Khan, A. Hameed,N. Abbas,A. Qayum and H.Inayat,2009. Antibacterial Activities of *Dodonaea viscosa* using Contact Bioautography Technique,Molecules, 14:1332-1341.

Kona, S and M. Rahman. 2016. Inventory of Medicinal Plants at Mahadebpur Upazila of Naogaon District, Bangladesh. *Applied Ecology Env. Sci*. 4, No. 3, 75-83.

Kozan, E., E. Küpeli and E. Yesilada, 2006. Evaluation of some plants used in Turkish folk medicine against parasitic infections for their *in vivo* anthelmintic activity,Journal of Ethnopharmacology, l108: 211–216.

Liu, H., N. Qiu,H. Ding and R. Yao, 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medicinal or food uses, Food Research International, 41(4): 363-370.

Mimica-Duki, N.M., V. Popovic, A. Jakovljevic, and O.G. Szabo,1999. Pharmacological studies of *Menthalongifolia*phenolics extracts. II. Hepatoprotective activity,Pharmaceutical Biology,l37: 221–224.

Mitscher, L.A.,G.S.R. Rao, T. Veysoglus, S. Drake,and T. Hass,1983. Isolation and identification of trachyloban -19-oic and (-)-kaur-16-en-19-oic acids as antimicrobial agents from the prairie sunflower, *Helianthus annus.*Journal of Natural Products, 46(5): 745.

Nair, R., V. Sumitra, Chanda, 2007. Antibacterial Activities of Some Medicinal Plants of the WesternRegion of India,Turkish Journal of Biology,31: 231-236.

Nundkumar, N. and J.A.Ojewole, 2002. Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine, Clinical Pharmacology,24(7): 397-401.

Okeke MJ, Iroeghu CU, Eze EN, Okoli AS, Esimone CO, 2001. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity, Journal of Ethanopharmacology, 78, 119-127.

Rahman, K., Muhammad Nisar, Amin Ullah Jan, Muhammad Suliman, Ajmal Iqbal, Ayaz Ahmad, Rukhsana Ghaffar, 2015. 'Antibacterial activity of important medicinal plants on human pathogenic bacteria. "International Journal of Agronomy and Agricultural Research,6 (6):106-111.

Samuelsson, G, 2004. Drug of natural origin: a textbook of pharmacognosy,5th Swidish Pharmaceutical Press. Stockholm.

Sanches,N. R., D.A.G. Cortez,M.S. Schiavini,C.V. Nakamura,B.P.D. Filho, 2005. An evaluation of antibacterial activities of *Psidium guajava* (L.). Brazilian Archives of Biology and Technology,48(3): 429–436.

Sharma, D., A.A. Lavania and A.Sharma, 2009.*In vitro* Comparative Screening of Antibacterial and Antifungal Activities of Some Common Plants and Weeds Extracts. Asian Journal of Experimental Sciences,23(1): 169-172.

Siddhuraju, P., K. Becker, 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L.). Food Chemistry, 101: 10-19.

Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal plants an expanding role in development. World Bank Technical Paper PP. 320.

Vyas, N., M. Tailang, N.P. Gavatia, and B.K. Gupta, 2010. Antioxidant Potential of *Psidium guajava* L. International Journal of Pharma Tech Research CODEN (USA), 2(1): 417-419.

World Health Organization, 2001. General guidelines for methodologies on research and evaluation of traditional medicine. WHO,Geneva, Switzerland., pp: 74.