

Phytochemical screening and antibacterial potential of *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus*

Rimsha Dilshad, Rida Batool and Nazia Jamil

Department of Microbiology and Molecular Genetics, Quaid-e Azam Campus, University of the Punjab, Lahore, Pakistan

Abstract: Utilization of herbs for medicinal purpose started in the early history of mankind several thousand years ago. In this study, some plants that are used for lowering cholesterol level in local areas of Pakistan, such as *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus* were screened for their phytochemical and antibacterial properties. For this purpose, these plants were extracted in different solvents i.e. ethanol, hexane and ethyl acetate. Phytochemical analysis unveiled the existence of different bioactive compounds in these extracts. Presence of sugars was further confirmed by performing TLC. Antibacterial activity was determined against indicated bacterial strains, among all extracts Gul-e-mundi had maximum inhibition zone (23mm). DPPH free radical assay revealed the significant antioxidative potential of all the extracts where Gul-e-mundi showed maximum potential i.e., 83%. Plant extracts were also showing anti-proliferative activity on root tips of *Allium cepa* and Gul-e-mundi was observed to have maximum antimitotic activity i.e. 5%. GC-MS analysis revealed that oleic acid and linoleic acid were the compounds responsible for imparting antibacterial potential to Gul-e-mundi. In conclusion, among all the tested extracts Gul-e-mundi had maximum antibacterial, antioxidative and antimitotic potential. For future studies, phytochemicals responsible for these activities can be isolated and modified for pharmacological purpose.

Keywords: Antibacterial activity, antimitotic activity, antioxidative activity, gas chromatography mass spectrophotometry, thin layer chromatography.

INTRODUCTION

Evidences unveil the reality that humans are well-known for utilizing animals and plants for medicine, food, wood or clothing since primitive times (Arshad *et al.*, 2014). A discipline known as “Ethnobiology” tells us about the relationship among living organisms and human cultures. These relationship studies can be prehistoric, historic or existing (Weber and Belcher, 2003). Ethnobiology is a broader term which consists of ethnobotany and ethnozoology as its disciplines. The word ethnobotany was first given by J. W. Harshberger as “the study of utilitarian relationship between human beings and vegetation in their environment, including medicinal uses” (Harshberger, 1896). Of about 250,000 - 500,000 plant species present on earth, only a small portion i.e. 1-10% is known to be used by humans and animals for food (Borris, 1996). Synthetic drugs have come into existence only few decades back in 1869 (Jones, 2011). Mostly these drugs are carbon copies of chemicals identified in plants i.e., phytochemicals (Junaid *et al.*, 2006).

Pakistan has a wide-ranging climate and loaded with medicinal herb, spread over a large area. Of these plants, about 600 plants are considered to have medicinal importance (Shinwari and Khan, 2000). In Pakistan, herbal medicines are used by local physicians known as hakims in herbal medicine centers (Tibbi Dawakhana). In Ayurvedic system of herbal treatment, plants are being

used commercially to extract their bioactive components for medicinal purposes (Mahmood *et al.*, 2004).

Infectious diseases are foremost cause of deaths globally (Westh *et al.*, 2004). But resistance to synthetic drugs (used for treatment) is emerging in pathogens due to the overuse of drugs (Service, 1995). This increase in resistance to the drugs has made pathogens, multiple drug resistant or extreme drug resistant (Bandow *et al.*, 2003). Other than the development of resistance, these synthetic drugs also pose some side effect on patients such as allergy, hypersensitivity or immune-suppression (Ahmad *et al.*, 1998). So to avoid such problems, there is a necessity of developing some new potential therapeutic means (Bhavnani and Ballow, 2000). These therapeutic agents must come from a source that is not harmful for the users and has lesser side effects (Cordell, 2000). That's why; researchers have diverted their focus towards folk medicine to treat infectious diseases (Benkeblia, 2004). Hunt for antimicrobial agents in different plants have begun in different parts of the world. Secondary metabolites are the major cause of antimicrobial effect of plants. These secondary metabolites may include alkaloids, phenols, steroids and tannins. These compounds are produced in either one part of a plant or in all the parts (Balandrin *et al.*, 1985). The function of phytochemicals in plants is affected by their unique grouping in different taxonomic groups. Same combination of these phytochemicals or bioactive compounds is not found in all plants. Therefore, their medicinal properties differ from one another (Wink, 1999). Secondary metabolites

*Corresponding author: e-mail: ridazaidi_1@yahoo.com

wield their antimicrobial action by mimicking endogenous metabolites of the human cells such as their hormones, ligands, signaling molecules or neurotransmitters (Principe, 1985). Plant derived extracts having antimicrobial potential must be tested against an appropriate microbial model so that its efficiency and other related parameters can be determined properly (Nair *et al.*, 2005). Initial stages of search for potential antimicrobial compounds should analyze crude plant extracts rather than pure compounds obtained from plants (Kusumoto *et al.*, 1995).

Artemisia absinthium (afsanteen) belongs to family Asteraceae. Artemisia is among the largest genera of this family being used for medicinal purposes (Juteau *et al.*, 2003). *Artemisia absinthium* is commonly known as Afsanteen or wormwood. The main constituents of its oil are absinthol, absinthin and anabsinthin, where later two are involved in providing bitter taste to the plant. Wormwood is effective in treating stomach and gall bladder problems (Weiss, 2001).

Swertia chirayita is commonly known as chirayita. This plant is known to contain antibacterial, antifungal, antihelmintic, antipyretic and hypoglycemic activities due to the presence of amarogentin, swerchirin, swertiamarin and other constituents (Handa and Kaul, 1996). Chirayita is known to have medicinal potential in all of its parts but the root is considered to be more important (Basu *et al.*, 1999). The thermal activity of this plant has been reported to be cooling. So, due to this property it is used to drain heat from blood and liver. It is said to be easily digestible and dry (Joshi, 2000).

Sphaeranthus indicus (Gul-e-mundi) belongs to Asteraceae family. This plant can be used as a whole for medicinal purposes because all of its parts have medicinal value. In ayurveda or folk medicine, it is mainly used to treat epileptic convulsions, mental illness, jaundice, diabetes, fever, cough, hernia, gastrophyl, skin diseases, hypercholesterolemia and different other diseases (Kirtikar *et al.*, 1987). Root of this plant is used to prepare oil from it which is useful in treating aphrodisiac and scrofula that is an infection caused by *Mycobacterium tuberculosis* (Ambavade *et al.*, 2006). A paste is also made from this plant which is useful in treating itching and arthritis, filariasis (round worm caused infection), gout, edema and cervical adenopathy. This plant is also used for treating hepatitis and piles (Paranjpe, 2001).

Afsanteen, Chirayita and Gul-e-mundi are locally used for the cure of hypercholesterolemia; so objective of the current study was to identify the phytochemical constituents and antibacterial potential of these plants. There is a need to explore phytochemicals in medicinal plants that are responsible for imparting them these medicinal characters. These identified nutrients can then be utilized in pharmaceutical and other industries to get

better and novel synthetic drugs. Herbal medicines can also help to develop a cheaper medication system.

MATERIALS AND METHODS

Collection of medicinal plants

Plants with the ability to lower cholesterol level in body were collected from a local area in Lahore, Pakistan. These plants included *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus*. Leaves and flowers of these plants were taken for the study.

Table 1: Selected medicinal plants

Plant	Selected Plant	Common Name	Plant Part
A	<i>Artemisia absinthium</i> L.	Afsanteen	Leaves
B	<i>Swertia chirayita</i>	Chirayita	Leaves
C	<i>Sphaeranthus indicus</i>	Gul-e-mundi	Flowers

Preparation of plant extracts

Medicinal plants were taken and washed under tap water to remove dust. These plants were air dried properly and crushed in to powder. Extracts of these plants were made by putting 10g of each plant material in 100ml of 3 solvents *i.e.* ethanol, hexane and ethyl acetate for 24 hours in dark. The extract was filtered using Whatman filter paper No1. This filtrate was then evaporated using Hei-vap-series, Heidolph Germany rotary evaporator.

Phytochemical analysis of selected medicinal plant extracts

Plant extracts selected for this study were tested for the bioactive compounds present in them.

Alkaloids

In a test tube, 1ml of the extract and 1ml of 1% HCl were mixed. This tube was then put in water bath at boiling temperature. Upon addition of 1ml of Wagner's reagent to this mixture, production of red precipitates indicated alkaloid's presence.

Carbohydrates

Carbohydrates were analyzed in the extracts by mixing 1ml of Molisch's reagent with 1ml of extract. One ml of concentrated H₂SO₄ was further added to it and left to stay for few minutes. After few minutes, red or violet color ring formation at the interface of two layers was observed (Ugochukwu *et al.*, 2013).

Cardiac glycosides

In 1 ml of extract, 0.5ml of glacial acetic acid was added along with a drop of ferric chloride (FeCl₃) solution in a test tube. Upon addition of 0.5ml of conc. H₂SO₄ to this

mixture, a brown ring was developed at the interface that indicated cardiac glycosides in extracts (Ugochukwu *et al.*, 2013).

Flavonoids

Half ml of 20% sodium hydroxide solution was mixed with 1ml of extract. The positive test was indicated by yellow coloration that became colorless upon addition of dilute hydrochloric acid (Ugochukwu *et al.*, 2013).

Phenols

Appearance of deep blue or black color when 0.5ml of 5% ferric chloride solution was mixed with 1ml of extract indicated the presence of phenols (Ugochukwu *et al.*, 2013).

Phlobatannins

Phlobatannins were tested in extracts by putting a mixture containing 1 ml of extract and 1ml of 1% HCl in boiling water bath. Red precipitates formation indicated the presence of phlobatannins (Kavit *et al.*, 2013).

Reducing sugars

One milliliter extract was dissolved in 1ml of distilled water in a tube. In another tube, 1ml of Fehling Solution A and B each were added and boiled in water bath. This solution was poured in extract and color change was observed as a positive test indication

Saponins

Saponins presence in extracts was indicated by the stable foam formation when 1ml of extract and 1ml of distilled water were mixed and shaken vigorously

Steroids

One ml of chloroform was mixed with 1ml of extract and 1ml of conc. H₂SO₄. Production of red color in the lower layer of chloroform indicated the presence of steroids (Kavit *et al.*, 2013).

Tannins

One milliliter of extract was added in 1ml of distilled water followed by a few drops of ferric chloride solution. Green colored precipitates indicated the existence of tannins in extracts (Kavit *et al.*, 2013).

Terpenoids

One milliliter of extract was mixed in one ml of chloroform and was left to evaporate. One ml of H₂SO₄ (concentrated) was then added and the mixture was heated for 2 minutes. Formation of grey color indicated the presence of terpenoids in extracts (Kavit *et al.*, 2013).

Estimation of antibacterial activity of medicinal plant extracts by agar well diffusion method

Agar well diffusion method is a qualitative assay that is used to determine the presence or absence of any

compounds with antibacterial activity (Valgas *et al.*, 2007). Mueller Hinton agar was prepared and autoclaved. On the agar plates, lawn of 24 hours old bacterial culture was made. A Pasteur pipette was then taken and sterilized and wells of 6mm diameter were made in the plates using it. Fifty microliter of the extract was poured in these wells along with respective negative controls. These plates were placed in an incubator at 37° C overnight. Afterwards, zones of inhibition (mm) shown by these extracts were measured.

Antioxidative assay of selected medicinal plant extracts

2,2-diphenyl-2-picryl hydrazyl (DPPH) is a free radical that changes its color from purple to yellow whenever it comes in contact with an antioxidant. A stock solution of 24mg DPPH per ml of methanol was prepared and stored at -20° C. With the help of this stock, working solution of absorbance 0.98±0.2 at 517nm was made. Three ml of the working solution was mixed with 100µl of sample and was incubated in dark for about 30 minutes. After incubation, absorbance of these mixtures was taken at 517nm. The percent radical scavenging activity (%RSA) or the percent antioxidant activity of these extracts was estimated by using following formula:

$$\% \text{Antioxidant Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Antimitotic activity of selected medicinal plant extracts

Good quality *Allium cepa* bulbs were taken from a local shop in Lahore. The outer coverings and the brownish base plate of the onions was removed and washed. Onion bulbs were transferred to glass bottles with tap water to let their roots grow up to 3cm for 3-5 days at room temperature in dark. The water in bottles was changed regularly after 24 hours. When the roots reached the desired length, extract was added to the water (diluting factor) in a ratio of 1:5. This treatment was continued for 1 day at room temperature in dark. The roots were plucked from the bulbs and were washed with tap water. These washed roots were put in the fixative for 24 hours. After incubation, these roots were washed and put in preservative (70% ethanol) at refrigerated temperature for future use. The roots were taken out of the refrigerator and washed with tap water. These roots were then put in an eppendorf with 1N HCl and were incubated for 5 minutes in water bath at 55-60° C for activating the roots. The activated roots were placed in a watch glass containing acetocarmine stain for 30 minutes. The stained roots were washed and the root tip was cut and placed over a glass slide. With the help of a syringe needle, the root tip was smashed to separate the cells so that overlapping of the cells can be avoided. A cover slip was placed over the cells and the slide was observed under light microscope.

The mitotic index of the cells was identified by counting the number of dividing and non dividing cells and putting them in following formula:

$$\text{Mitoticindex} = \frac{\text{Number of dividing cells}}{\text{Total Number of cells}} \times 100$$

Thin layer chromatography (TLC)

Thin layer chromatography is a technique that can be used to separate different components present in a crude extract. The targeted components were sugars, glucosides and some other components present in the extracts. Mobile phase used for this separation consisted of chloroform, methanol and water in a ratio of 65:35:10. The plates were developed in TLC tank. When the solvent reached 1 inch less than the entire TLC plate length, the plate was removed from the tank and allowed to air dry. The plate was observed under high and low range Ultra Violet rays and the spots were marked with a lead pencil. Plate was placed in iodine for a few seconds and iodine activated compounds were observed and marked. After these observations the plate was covered with 10% H₂SO₄ and put in oven at 120°C for 10 minutes. This spray revealed brown spots on the plate that showed the presence of sugar and glycosides in the extracts. Ethyl acetate extract plate was developed in chloroform only as the mobile phase. The plate was viewed and marked under high and low ultraviolet rays and iodine.

Gas chromatography mass spectrophotometry (GC-MS)

All of the selected extracts showed bacterial inhibition but maximum zone size was observed for *Sphaeranthus indicus* ethyl acetate extract i.e. 15mm. This extract was then further analyzed for the bioactive component responsible for this antibacterial activity.

TLC

Crude extract of *Sphaeranthus indicus* flowers was spotted on a line, 1.5cm above the base of the TLC plate. This plate was developed in chloroform (solvent system). The separated components were marked under short and long UV rays and iodine. Observed spots were marked from 1 to 8 and R_f values were calculated.

Extracting components from TLC plate

Marked spots were scratched from the TLC plate in respective tubes containing ethyl acetate to dissolve the extract components in it. After 24 hours, the extracts from TLC plate were filtered and the filtrate was dried at room temperature.

Antibacterial activity of selected spots

Antibacterial activity of these spots was determined against a gram positive strain. Bacterial lawn was formed on two MH-agar plates. Discs were made from Whatman filter paper no. 1 and were autoclaved. These discs were dipped in 100µl extract for 5 minutes so that partially purified extract spot could get adhered to the disc. Respective discs were then air dried near flame and positioned on the plate with a negative control i.e. ethyl acetate disc, on each plate. The plates were put in

incubator for 24 hours at 37°C. After incubation, zone of inhibition was measured against each component.

GC-MS

Gas chromatography mass spectrophotometry (GC-MS) analysis of the spot number 6 that had antibacterial activity was done. Column length of spectrophotometer was 30m using helium as a carrier gas. Temperature of injection port was kept at 230°C and 1µl of sample was injected with flow rate of compound as 1ml/min. The rate of temperature change was maintained at 5°C/min. with initial temperature at 100°C and final temperature at 270°C. The column was run for about 35 minutes and peaks were observed.

RESULTS

Phytochemical Analysis of selected medicinal plant extracts

Medicinal plants used for the treatment of several diseases mainly hypercholesterolemia, were collected from Lahore, Pakistan. To check the bioactive compounds in the selected plant extracts, different phytochemical tests were done. The results obtained for these tests are given in table 2.

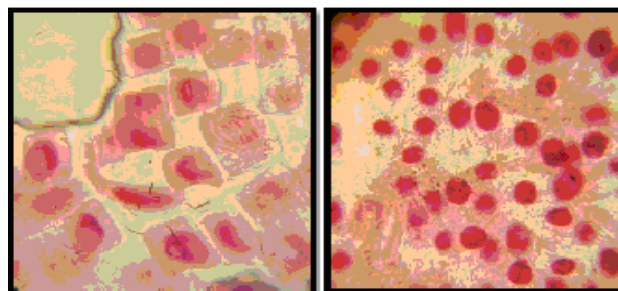


Fig. 1: Left: Root cells grown in simple tap water. Right: Gul-e-mundi extract treated root cells showed antimittotic activity



Fig. 2: TLC plate showing ethanol extract components separated by developing the plate in Chloroform:

Table 2: Phytochemical analysis, antibacterial, antimutic and antioxidative activity of selected medicinal plant extracts.

Extracts	Phytochemical tests											Antibacterial Activity (mm)		Antimutic activity (%)	Antioxidative activity (%)
	Alkaloids	Carbohydrates	Cardiac Glycosides	Flavonoids	Phenols	Phlobatanins	Reducing sugars	Saponins	Steroids	Tannins	Terpenoids	Gram Positive bacteria	Gram Negative bacteria		
Ethanol	N	-	-	-	-	-	-	-	-	-	-	02±0.08	05±0.06	45.0±0.80	05.0±0.03
	A	+	+	+	+	-	+	-	+	-	+	12±0.12	11±0.22	25.8±0.72	66.2±0.04
	B	-	+	-	-	-	+	-	-	-	+	24±0.44	13±0.25	23.0±0.41	69.3±0.08
	C	+	+	+	+	+	+	-	-	+	+	19±0.59	23±0.12	16.6±0.83	83.2±0.02
Hexane	N	-	-	-	-	-	-	-	-	-	-	00±0.00	00±0.00	42.0±0.50	03.3±0.02
	A	-	+	+	+	-	-	-	-	-	+	02±0.68	05±0.53	07.5±0.91	0.30±0.07
	B	-	+	+	+	-	-	-	-	-	+	04±0.89	01±0.35	40.0±0.82	04.7±0.02
	C	-	+	+	-	-	-	-	+	-	+	17±0.44	14±0.17	05.0±0.72	03.5±0.02
Ethyl Acetate	N	-	-	-	-	-	-	-	-	-	-	08±0.50	01±0.07	40.0±0.43	04.2±0.04
	A	+	+	+	+	-	+	-	+	-	+	21±0.35	04±0.70	15.0±0.52	54.6±0.06
	B	-	+	+	+	-	-	-	+	-	-	13±0.03	13±0.53	18.0±0.37	48.7±0.07
	C	-	+	+	+	-	-	-	-	-	-	23±0.17	03±0.35	10.0±0.63	83.4±0.05

Mean of three replicates, ± Standard error of mean, Where, N= Control, A= Afsanteen, B= Chirayita, C= Gul e Mundi

methanol: water (65:35:10) solvent system after iodine spray.

Antibacterial activity

Property of any compound to inhibit bacterial growth is called antibacterial activity of the compound. This activity of selected plants was determined using agar well diffusion method. Antibacterial activity of these extracts was tested against a gram positive strain (JQ013099) and a gram negative strain (W3 from MMG bacterial stock culture). All the extracts variably repressed the growth of bacterial strains as given in table 2.

Antimutic activity

Results revealed that Gul-e-mundi plant had maximum antimutic activity in all the three extracts i.e. ethanol, hexane and ethyl acetate. The activity of Gul e mundi ethanol, hexane and ethyl acetate extracts was 16.6%, 5% and 10%, respectively. Antimutic activity of other extracts is given in table 2.

Antioxidative assay

Maximum antioxidation capacity was observed for Gul-e-mundi in ethanol and ethyl acetate extracts, while in hexane extracts maximum capacity was shown by Chirayita. The activity of other extracts is given in table 2.

Thin layer chromatography

Sphaeranthus indicus was found to have maximum antibacterial, antioxidative and antimutic potential that’s why this plant was chosen to analyze the components responsible for its antibacterial potential. A spot of

Sphaeranthus indicus ethyl acetate extract was placed on a TLC plate and was run in chloroform. Eight spots were separated from this crude extracts. All of these components were checked for their antibacterial activity. Only spot 6, with Rf value 0.27, was positive for bacterial growth inhibition that gave 2mm inhibition zone.

GC-MS

GC-MS is a chromatographic technique used to identify, separate or quantify the compounds present in a mixture. The purpose of GC-MS analysis in this study was to identify the partially purified bioactive compound that possessed antibacterial activity in *Sphaeranthus indicus* ethyl acetate extract, on the basis of its mass. Analysis of this compound gave two peaks at 25.33 and 32.36 minutes retention time. Mass analysis of these compounds revealed them to be oleic acid and erucic acid, respectively.

DISCUSSION

Use of plants for medicinal purposes started about 60,000 years ago (Sullivan et al., 2010). Plants have acquired their medicinal properties due to the bioactive compounds present in them (Edeoga et al., 2005). Several studies revealed that these medicinal plant extracts can be used for the discovery of novel medicines as the need for medicines is increasing day by day (Balunas and Kinghorn, 2005).

In present study, *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus* were targeted to

identify their phytochemical constituents responsible for their antibacterial, antimutagenic and antioxidative ability in addition to local use of these plants for lowering the cholesterol level.

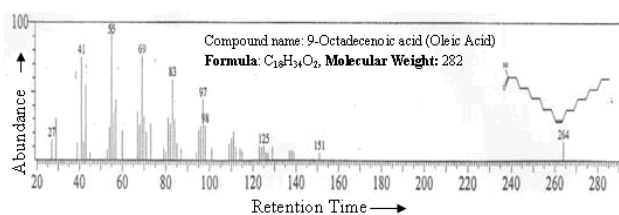


Fig. 3: Mass spectrum of oleic acid and its structure.

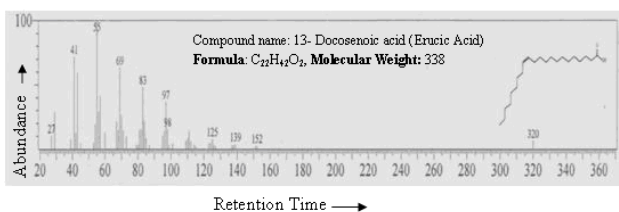


Fig. 4: Mass spectrum of erucic acid and its structure.

Presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, phlobatannins, reducing sugars, phenols, saponins, tannins, steroids and terpenoids was confirmed in these medicinal plants by phytochemical analysis. The chemical composition of crude extract also varied from one solvent to the other. Ethanol extracts were positive for most of the bioactive compounds while hexane and ethyl acetate extracts were positive for lesser phytochemical constituents. This difference in the composition of extracts can be attributed to the fact that different solvents have the potential to dissolve various compounds depending upon the polarity of solvent (Argolo *et al.*, 2004). Ethanol has the highest polarity among selected three solvents, while hexane has the minimum polarity that is why ethanol has the ability to dissolve most of the bioactive constituents of plants in it rather than the other solvents (Dong and Winnik, 1984).

Some phytochemicals like carbohydrates, cardiac glycosides and terpenoids were found in almost all the extracts of selected plants whereas alkaloids, phenols, phlobatannins, and tannins were present in only a few of the extracts. While, according to Harborne 1998, alkaloids, flavonoids, terpenoids, phlobatannins, and tannins are present in most of the plants (Harborne, 1998).

The antibacterial activity of extracts was estimated against a gram positive and a negative strain. All of the strains showed bacterial inhibition. It shows all of the extracts have potent antibacterial components in them. Maximum inhibition against both the strains was observed for ethanol extract followed by ethyl acetate. While, minimum inhibition potential was observed for hexane extracts. The highest bacterial growth inhibition potential of ethanol can be due to the reason that bioactive

compounds for bacterial inhibition were able to dissolve lesser in non-polar solvent while more in polar solvents as a result of which ethanol had maximum antibacterial activity (Henie *et al.*, 2009).

All the extracts of ethyl acetate, hexane and ethanol showed greater inhibition activity for gram positive strains while inhibition of gram negative strains was lesser. This difference in inhibition of gram positive and negative strains is because gram negative bacteria have an extra layer surrounding them which makes them hard to be targeted by the compounds having antibacterial activity (Smith *et al.*, 1998). Bigger zone of inhibition could be due to the flavonoids or tannin content in the extracts as they are reported to have high antibacterial potential. Whereas, smaller zone of inhibition of extracts could be because of the lesser penetration potential of extracts, through the agar, to inhibit the growth of bacteria (James, 2007).

The maximum inhibition of bacterial growth was shown by ethanol extracts is in accordance with the previous studies of Ijeh *et al* (2005) and Junaid *et al* (2006). According to them, the antibacterial potential of any extract is directly related to the solubility of antibacterial compounds in solvent. This solubility is dependent on the solvent polarity and on this basis they have characterized alcohol as the best solvent for extraction (Junaid *et al.*, 2006).

Recent studies have revealed that phytochemicals are the plant constituents that help in protecting the body from oxidative stress induced diseases. Free radicals are released in the body due to oxidative stress. The free radicals harm the body causing certain diseases such as cardiac diseases, cataracts and cancer (Kaur and Kapoor, 2001). Antioxidants in plants scavenge the free radicals in the body thus reducing the effect of the diseases caused by oxidative stress (Agbafor and Nwachukwu, 2011).

DPPH (2, 2- diphenyl 1-1 picrylhydrazyl) was used as a free radical to check the presence of antioxidants in extracts. Ethanol and ethyl acetate extracts had high potential for antioxidation while hexane did not show any significant antioxidation. This increased antioxidation potential of ethanol and ethyl acetate can be due to the phenolic compounds like flavonoids in the extracts as these components are potent antioxidants (Jayaprakasha *et al.*, 2008). Hexane extracts showed minimum activity *i.e.* they had lesser concentration of phenols present in them that are usually responsible for antioxidation. This result is similar to the findings of Rzesutek and Chow, (1998) who suggested that phenols are less soluble in hexane hence; they may have lesser antioxidation ability.

There are certain agents that have the capacity to disturb mitosis in the cells by disrupting the mitosis at any point of cell cycle; these agents are called anti mitotic agent

(Akrai and Abdulrahman, 2013). In this study, all the extracts showed significant antimitotic activity. The maximum antimitotic activity was observed by ethanol and hexane extracts of Gul-e-mundi i.e., *Sphaeranthus indicus*. The reason for the extracts to show high antimitotic activity can be the amount of glycosides present in the extracts. These glycosides may include anthraquinones and phenolic compounds that may bind to the cellular proteins that mediate the cell cycle or cell division (Akrai and Abdulrahman, 2013).

This antimitotic activity of plant extracts can contribute in identifying drugs for cancer treatment. As in cancer, the cells tend to multiply rapidly through mitosis. The antimitotic agents hinder their proliferation by interfering in the cell cycle kinetics. These antimitotic agents may pose harm to the normally dividing cells so the choice of drug for this purpose must be focused on an extract that has lesser side effects (Bhujbal *et al.*, 2011).

The test system used in this study was *Allium cepa* because, the chromosomal aberrations that are produced by any antimitotic agent on plant chromosomes is strikingly similar to the aberrations shown by mammalian cells (Auti *et al.*, 2010). Plant systems especially the root tips are well known for studying the chromosomal aberrations. This method is the oldest, simplest and the most reliable one for determining genetic damages to the cells. Other than these advantages, this method is also very economical (Grant, 1978).

Thin layer chromatography (TLC) technique was performed to separate the different components present in extracts. All the extracts were separated into different bands showing the presence of different compounds in those extracts. A brown band was observed in each of the spot of ethanol and hexane extracts. The brown spot was an indication of sugars present in the ethanol and hexane extracts as reported by Chavan and Amarowicz, 2013.

GC-MS analysis was done to find the bioactive compound in *Sphaeranthus indicus* that is responsible for antibacterial activity of this plant. The antibacterial activity observed by this spot was lesser than the activity of whole plant. This could be attributed to lesser concentration of the component and might also be due to the synergistic effect of whole plant components. Two components were identified via GC-MS analysis; of which Oleic acid is a bioactive component that is reported to be present in *Sphaeranthus indicus* and is involved in larvicidal action of the plant extract (Rahuman *et al.*, 2008). While, erucic acid is not found in *Sphaeranthus indicus* but it is present in rapeseed oil (Harvey and Downey, 1964). Erucic acid is also reported to have antimicrobial activity in plants (Bailey *et al.*, 1989).

CONCLUSION

The cholesterol lowering medicinal plants *Artemisia absinthium* L., *Swertia chirayita* and *Sphaeranthus indicus* used in this study were found to have antimicrobial, antioxidant and antimitotic potential due to the presence of certain phytochemicals in them. These potential phytochemicals can prove to be pharmacologically beneficial because these phytochemicals can be isolated from crude extracts for manufacturing new synthetic drugs. Future studies should be focused on the analysis of phytochemical constituents of cholesterol lowering medicinal plants. Phytochemicals responsible for lowering the cholesterol level should be isolated and modified for pharmacological purpose.

ACKNOWLEDGEMENT

Authors are thankful to University of the Punjab, Lahore for providing financial assistance for the accomplishment of this research work.

REFERENCES

- Agbafor KN and Nwachukwu N (2011). Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. *Biochem Res Int.*, doi:10.1155/2011/459839.
- Ahmad I Mehmood Z and Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, **62**(2): 183-193.
- Akrai HFS and Abdulrahman ZFA (2013). Evaluation of the antibacterial efficacy and the phytochemical analysis of some plant extracts against human pathogenic bacteria. *Journal of Pharmacology and Clinical Sciences*, **7**: 29-39.
- Ambavade S, Mhetre N, Tate V and Bodhankar S (2006). Pharmacological evaluation of the extracts of *Sphaeranthus indicus* flowers on anxiolytic activity in mice. *Indian J. Pharmacol.*, **38**(4): 254-259.
- Argolo ACC, Sant'Ana AEG, Pletsch M and Coelho L (2004). Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresour Technol.*, **95**(2): 229-233.
- Arshad M, Ahmad M, Ahmed E, Saboor A, Abbas A and Sadiq S (2014). An ethnobiological study in Kala Chitta hills of Pothwar region, Pakistan: multinomial logit specification. *J. Ethnobiol. Ethnomed.*, **10**: 13.
- Auti S, Pagare R, Ahire D and Sawale V (2010). Cytogenetical studies on the effect of omnacortil on root tip cells of *Allium cepa* L. *Cell Tissue Res.*, **10**(3): 2331-2335.
- Bailey AV, De Lucca AJ and Moreau JP (1989). Antimicrobial properties of some erucic acid-glycolic acid derivatives *J. Am. Oil Chem. Soc.*, **66**(7): 932-934.

- Balandrin MF, Klocke JA, Wurtele ES and Bollinger WH (1985). Natural plant chemicals: Sources of industrial and medicinal materials. *Science*, **228**(4704): 1154-1160.
- Balunas MJ and Kinghorn AD (2005). Drug discovery from medicinal plants. *Life Sciences*, **78**(5): 431-441.
- Bandow JE, Brötz H, Leichert LIO, Labischinski H and Hecker M (2003). Proteomic approach to understanding antibiotic action. *Antimicrob. Agents Chemother.*, **47**(3): 948-955.
- Basu BD, Kirtikar K and Blatter E (1999). Indian medicinal plants: International Book Distributors.
- Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT - Food Science and Technology*, **37**(2): 263-268.
- Bhavnani SM and Ballow CH (2000). New agents for Gram-positive bacteria. *Curr. Opin. Microbiol.*, **3**(5): 528-534.
- Bhujbal SS, Deshmukh RP, Bidkar JS, Thatte VA, Awasare SS and Garg PP (2011). Evaluation of cytotoxic activity of barks of *Mimusops elengi*. *EurAsian Journal of Bio Sciences*, pp.73-79.
- Borris RP (1996). Natural products research: perspectives from a major pharmaceutical company. *J. Ethnopharmacol.*, **51**(1): 29-38.
- Chavan U and Amarowicz R (2013). Effect of various solvent systems on extraction of phenolics, tannins and sugars from beach pea (*Lathyrus maritimus* L.). *Int. Food Res. J.*, **20**(3): 1139-1144.
- Cordell GA (2000). Biodiversity and drug discovery a symbiotic relationship. *Phytochemistry*, **55**(6): 463-480.
- Dong DC and Winnik MA (1984). The Py scale of solvent polarities. *Can. J. Chem*, **62**(11), 2560-2565.
- Edeoga H, Okwu D and Mbaebie B (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.*, **4**(7): 685-688.
- Grant WF (1978). Chromosome aberrations in plants as a monitoring system. *Environ. Health Perspect.*, **27**: 37-43.
- Handa S and Kaul MK (1996). Supplement to cultivation and utilization of medicinal plants (6th ed.): Regional Research Laboratory, Council of Scientific & Industrial Research, Jammu-Tawi.
- Harborne JB (1998). Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media.
- Harshberger J. W. (1896). The purposes of ethno-botany. *Botanical Gazette*, pp.146-154.
- Harvey B and Downey R (1964). The inheritance of erucic acid content in rapeseed (*Brassica napus*). *Can. J. Plant Sci.*, **44**(1): 104-111.
- Henie E, Zaiton H and Suhaila M (2009). Bacterial membrane disruption in food pathogens by *Psidium guajava* leaf extracts. *Int. Food Res. J.*, **16**: 297-311.
- James D (2007). Phytochemical and antimicrobial investigation of the aqueous and methanol extracts of *Ximenia americana*. *Journal of Medical Sciences*, **7**(2): 284-288.
- Jayaprakasha GK, Girenavar B and Patil BS (2008). Radical scavenging activities of Rio red grapefruits and sour orange fruit extracts in different in vitro model systems. *Bioresour. Technol.*, **99**(10): 4484-4494.
- Jones AW (2011). Early drug discovery and the rise of pharmaceutical chemistry. *Drug Test Anal.*, **3**(6): 337-344.
- Joshi S. G. (2000). Medicinal plants. Oxford and IBH Publishing.
- Junaid S, Olabode A, Onwuliri F, Okwori A and Agina S (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afr. J. Biotechnol.*, **5**(22): 2315-2321.
- Juteau F, Jerkovic I, Masotti V, Milos M, Mastelic J, Bessiere JM and Viano J (2003). Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. *Planta Medica*, **69**(2): 158-161.
- Kaur C and Kapoor HC (2001). Antioxidants in fruits and vegetables the millennium's health. *Int. J. Food Sci. Technol.*, **36**(7): 703-725.
- Kavit M, Patel B and Jain B (2013). Phytochemical analysis of leaf extract of *Phyllanthus fraternus*. *Res. J. Recent Sci.*, **2**(1): 12-15.
- Kirtikar KR, Das Basu B, Blatter E, Caius JF and Mhaskar KS (1987). Indian Medicinal Plants. International Book Distributors. India.
- Kusumoto IT, Nakabayashi T, Kida H, Miyashiro H, Hattori M, Namba T and Shimotohno K (1995). Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. *Phytother. Res.*, **9**(3): 180-184.
- Mahmood A, Ahmad M, Jabeen A, Zafar M and Nadeem S (2005). Pharmacognostic studies of some indigenous medicinal plants of Pakistan. *Ethnobotanical Leaflets*, **9**: 1.
- Nair R, Kalariya T and Chanda S (2005). Antibacterial activity of some selected Indian medicinal flora. *Turkish J. Biol.*, **29**(2005): 41-47.
- Paranjpe P (2001). Indian medicinal plants: Forgotten healers: A guide to ayurvedic herbal medicine with identity, habitat, botany, photochemistry, ayurvedic properties, formulations & clinical usage, Vol. 26, Chaukhamba Sanskrit Pratishthan, India.
- Principe PP (1985). The economic significance of plants and their constituents as drugs, Vol. 3, Academic Press Limited.
- Rahuman AA, Venkatesan P and Gopalakrishnan G (2008). Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol. Res.*, **103**(6): 1383-1390.
- Service RF (1995). Antibiotics that resist resistance. *Science*, **270**(5237): 724-727.

- Shinwari MI and Khan MA (2000). Folk use of medicinal herbs of Margalla hills national park, Islamabad. *J. Ethnopharmacol.*, **69**(1): 45-56.
- Smith P, Stewart and Fyfe (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol.*, **26**(2):118-122.
- Sullivan R, Behncke I and Purushotham A (2010). Why do we love medicines so much? An evolutionary perspective on the human love of pills, potions and Placebo. *EMBO Rep.*, **11**(8): 572-578.
- Ugochukwu SC, Arukwe UI and Ifeanyi O (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G Baker. *Asian Journal of Plant Science and Research*, **3**(3):10-13.
- Valgas C, Souza SMD, Smânia EF and Smânia Jr A (2007). Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.*, **38**(2): 369-380.
- Wadood, A., Ghufran, M., Jamal, S., Naeem, M., Khan, A. and Ghaffar, R. 2013. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, **2**(4): 1-4.
- Weber SA and Belcher WR (2003). *Indus Ethnobiology*, Vol. 1, Lexington Books, New York.
- Weiss RF (2001). *Weiss's herbal medicine*, 6 ed., Thieme Medical Publishers, India.
- Westh H, Zinn CS, Rosdahl VT and Group SS (2004). An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb Drug Resist.*, **10**(2): 169-176.
- Wink M (1999). *Biochemistry of secondary product metabolism*. CRC Press, Florida.