Assessment of biological activities of *Myrsine africana* leaves and fruit

Hina gul [1], Samra irum [2], Sadia mehmood satti [1], Riffat Batool [1]

ABSTRACT

Traditional medicines have now turned out to be a burning topic for their beneficial effects. There is a growing concern for the compositional investigation of overlooked plants and phytochemicals are responsible for the medicinal properties of plants. In this study *Myrsine africana* was assessed for the antioxidant, antimicrobial, anti-hemolytic, anti-tuberculosis and anti-tumor activity. Results showed that this plant is rich in phytochemicals and antioxidant activity. Methanol extract of *Myrsine africana* leaves were found to be more effective as compared to other extracts. *Myrsine africana* showed good results in in-vitro studies so it could further be used for investigation in pharmacological and toxicological studies.

Key words: *Myrsine africana*: phytochemicals: biological activities

---

[1] University Institute of Biochemistry and Biotechnology PMAS Arid Agriculture Rawalpindi

[2] Department of Biological sciences, International Islamic University, H 10 campus, Islamabad

INTRODUCTION

There are various herbal formulates that possess beneficial effects on human health. The remarkable uses and less adversarial effects of medicinal plants with their significant bioactive compounds helps in the management of various human diseases (Nabavi et al., 2016). The use of traditional medicines has become a burning subject around the globe for its lesser side effects. There is a rising concern towards compositional screening of undiscovered plants and their derived products (Karakas et al., 2016).

The global problem regarding insufficient control of disease has occurred by the repeatedly use of commercial drugs which is responsible for the development of resistance against antimicrobial compounds or synthetic drugs. It compels the researchers to reveal plant derived

antimicrobial metabolite. Medicinal Plants and their associated natural products have been clinically reported as chemo preventive and antimicrobial agents (Hussain *et al*., 2016). MDR (Multiple drug resistance) has been developed due to uneven use of synthetic drugs for curing various infectious diseases. Furthermore, the antibiotic usage leads to multiple side effects on consumer health which includes allergy reactions, immune suppression and hypersensitivity. These alarming conditions turned out in a great need of valuable remedial products. Thus, it is necessary to generate antimicrobial drugs from the medicinal plants for curing various infectious diseases (Ramamurthy et al*.,* 2013: To et al., 2017).

Phyto-chemicals define the medicinal properties of plants and are categorized into qualitative and quantitative metabolites (phenolic, flavonoids, alkaloids, steroids, saponins and tannins). Among the active compounds, phenols and flavonoids are the most important groups and impart various pharmacological properties such as anti-oxidative, anticancer, anti-allergic, hypoglycemic, antimicrobial and hepato-protective activity (Li et al., 2017: Dib et al., 2017).

Reactive oxygen species (ROS) act with different bio-molecules i.e. carbohydrates, protein, lipids and DNA alter the cis in to trans fatty acids that is responsible for several chronic diseases i.e. chronic inflammations, heart diseases, cancer and aging (Yasir *et al.*,2016). Medicinal Plant constitutes natural antioxidants to scavenge free radicals through protecting cells from oxidative damage. Amongst antioxidants, great thought has been granted to the flavonoids and phenolic compounds which inhibits or slow down the oxidative stress and decreases the possibility for degenerative diseases (Stankovic, 2011).

METHODOLOGY

Sample preparation

Samples of *Myrsine africana* leaves and fruits were collected from some areas of Kotli sattian during March and April 2017. The collected samples were placed in fine plastic bags duly labeled with name, date and areas of collection. The samples were shade dried followed by sun and oven drying for overnight at 60º C. The dried samples were ground by using electrical grinder, sieve (80 meshes) and saved in the fine plastic bags for further uses.

The Plants samples were collected with the help of local informant and the samples were properly identified by expert taxonomist on the basis of previous literature, herbarium specimens and by different sites (Plant lists, flora of Pakistan). Cleaned specimens were subjected to drying followed

by pressing, poisoning and mounting of plant species and then submitted to herbarium of Pakistan (ISL) for the future record.

Preparation of plant powder

Take the desired parts of the plant sample followed by shade drying. Grind it to a fine powder and sieved. After sieving place the sample in heating oven at 37◦c to remove moisture for complete drying. After complete drying the powdered material was ready for the further analysis.

Preparation of Plant Extracts

Total 500 grams of samples were dissolved separately in methanol and chloroform. They were extracted by using Soxhlet apparatus and rotary evaporator techniques followed by shaking for overnight. Filtration of all the extracts was done so that the residue gets separated from the desired extracts. The dried extracts were stored in air tight vials for further processes.

Phytochemical analysis

For the quantification of TPC (total phenolic content), Folin-Ciocalteu reagent method was used and TPC (total flavonoids) were estimated by using the method of aluminium chloride (Ghasemzadeh, 2011).

Antioxidant Activity of Plants

DPPH Scavenging Activity

This method is assessed by using the modified protocol (Moon and Shibamoto, 2009). Different concentrations ranges from 20 to 100 µg/ml of plant sample along with 2ml of DPPH solution and left the mixture for 30 minutes in darkness. The reading was taken at 517nm. The activity was determined by the following formula

DPPH %= $[A^A - A^H/A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

Gallic acid was used as a Standard or Positive control. IC 50 was measured by linear regression analysis and defined as the 50% inhibition concentration of the extract.

Iron Chelating Assay

The method used is described by the (Dinis *et al*., 1994). The plant extracts ranged from 20 to100 µg/ml were used for the chelating of Fe 2+. Then add 1ml of 2mM ferrous sulphate and with the addition of 0.25mM Ferrozine (1ml) reaction was initiated. After shaking, left the mixture for10 minutes and at 517 nm absorbance was recorded.

Chelating rate %= $[A^A - A^H /A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

Gallic acid was used as a Standard.

Hydroxyl Radical Scavenging Assay

Plants extracts ranges from 20 to100 µg/ml were investigated by the deoxyribose method (Nagai *et al*., 2005). Add Sodium phosphate buffer (0.2M) of 7 pH followed by 2deoxyribose (10Mm), FeSO 4 -EDTA (10Mm), H 2 O 2 (10mM), 525µl of H 2 O. Then add the mixture of TCA (2.8%) and TBA (1%) in Na OH. Absorbance was observed at 520nm.

Gallic acid was used as Positive control.

Scavenging activity= $[1- A^H /A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

ABTS (2,2- azinobis [3- ethylbenzothiazoline-6- sulfonate]) Radical Cation Decolorisation Assay

The method used is described by (Ashafa et al., 2010). The mixture of 3mM (final concentration) ABTS (2,2- azinobis [3- ethylbenzothiazoline-6- sulfonate]) with water was oxidized by adding potassium persulfate that is 2.5Mm. Place this mixture in dark for 12 hours. After diluting this ABTS + solution with distilled water absorbance should be 2.51±0.05.The plant extracts ranged from 20 to100 µg/ml. Absorbance was measured at 734nm. Gallic acid was used as Standard.

Percent Scavenging potential = $[A^A - A^H /A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

Reducing Power Assay

FRAP (Ferric ion reducing power) value was measured (Adedapo *et al.*, 2009). Different concentration of plant samples ranged from 20 to100 µg/ml was taken with 0.2M phosphate buffer and potassium ferricyanide (0.1%). Incubate the mixture in water bath till 20 minutes. By adding trichloroacetic acid (10%) the above reaction was ended. The above layer of this solution was added in distilled water (2ml) followed by ferric chloride (0.01%) and incubated for 20 minutes. The readings of blank and samples were observed at 700nm. Gallic acid was used as positive control. The results were expressed as GAE (mg/g of compound extracted).

Hydrogen Peroxide Scavenging Activity (H_2O_2)

This activity was described as the method (Aiyegoro and Okoh, 2010). Add H_2O_2 solution 4Mm (preparation inphosphate buffer) indifferent plant concentrations followed by incubation for 10 minutes. The absorbance was observed at 230nm.

Scavenging activity% = $[A^A - A^H / A^A]*100$

A^A is the reaction mixture absorbance except plant extract.

A^H is the reaction mixture absorbance having plant extract.

Superoxide Assay

The assay was used with some modified procedure (Beauchamp and Fridovich, 1971). Add 50mM Phosphate buffer, Riboflavin, 20Mm PMS, 0.5Mm NBT in various concentrations of plant samples and incubate for 20 minutes. The absorbance was recorded at 560nm.The positive control was Gallic acid.

Scavenging percentage = $[1- A^H / A^A]*100$

A^A is the reaction mixture absorbance except plant extract. A^H is the reaction mixture absorbance having plant extract.

Determination of antibacterial activities of extracts

Microorganism Tested

The following bacteria *Echerichia coli* (ATCC15224), *klebsiella pneumonia* (MTCC618), *Salmonella gallinarum*, *Staphylococcus aureus* (ATCC 6538), *Microcrocus lotus*, *Enterobacterauregens*, *Bacillus brevis*, *Pseudomonas aeruginosa* were tested for antimicrobial activity by Well diffusion method. Inoculum of microbes was made in LauriaBrothgL- 1 and kept it in shaking incubator for 24hours (contained 10 8 cfu/ml) at 37 ºC.

Well diffusion Method

Antimicrobial activity was done by well diffusion method (Ettebong and Nwafor, 2009) with some modifications. Media contained Lauria-Bertini (LB) agar and autoclaved the agar (121 ºC) for 15 min. After cooling poured it in the Petri plates in laminar flow hood. Add 30 µl of inoculums in all the plates for inoculation. Then the disk is impregnated into the plates with sterilized forceps after dipping in the plant extracts (50 µl). Incubation of plates was done (37 ºC) for 24 hours. The inhibition zones were observed after 24 hours and presented in millimeter.

Minimum Inhibitory Concentration (MIC)

MIC (Minimum inhibitory concentration) of the plant samples was examined by tube dilution method in Lauria-Bertini broth media. Each dilution (3.125-50 mg/mL) was inoculatedwith 5 x 10 6 of the bacterial strain culture incubated for 24 h at 37ºC. The standard

drug (Gentamicine) was used as positive control at same concentration. The absorbance of the suspension was observed at 420 nm along with the blank. The MIC was measured as lowest concentration of plant extract that inhibits the test cultures with no observable growth (Hemandez-Hernandez et al., 2017).

Anti-tuberculosis activity

The two drug resistant strains of *Mycobacterium tuberculosis* bg 206 and bg 1972, while a sensitive strain H37Rv were obtained from National TB Reference Laboratory, National TB Control Program Chak-Shahzad, Islamabad. Lowenstein-Jensen (LJ) medium was used in this experiment for the growth of isolates. Plant extract was incorporated in to culture medium and set for incubation at 37ºC and for 40 days. Readings were taken in triplicate manner. Control was also taken and susceptibility of MDR strains was done against standard rifampicin. MIC is the lowest extract concentration and it was determined by using 96 well micro titer plates (Ishikawa *et al*., 2017).

Antihemolytic activity

The method reported by (Alinezhad *et al*., 2013) with some modification was used to determine anti-hemolytic activates of plant extracts. The Methanol plant extracts were assessed against Human erythrocytes and absorbance was measured at 640 nm by using UV-Spectrophotometer and described as

Antihemolytic activity %= Control- Sample/Control*100.

Antitumor activity

Potato disc assay was adopted as described by (Hussain *et al*., 2007), and results were described as

Percentage inhibition (%) = 100- Number of tumors per sample/ Number of tumors in control*100.

STATISTICAL ANALYSIS

All the results were obtained in triplicate manners with mean and standard deviation.

RESULTS AND DISCUSSION

Among secondary metabolites phenolic compounds are considered the most important compounds and found to be present in more quantity as compared to other compounds. Methanol extract of *c* have higher quantity of phenols 362±9 mg/g of dry weight followed by 358.4±5 in *Myrsine africana* fruit While chloroform extracts of *Myrsine africana* leaves and fruits have 340.5±1 and 320±3. Phenols were present in higher quantity as compared to other phytochemicals and quantified in the current study. Phenols impart various pharmacological properties and effective

against different diseases e.g. anticancer activity, cardiovascular diseases, rheumatism. After phenols, flavonoids are the second to be found in higher quantity as compared to other secondary metabolites. Flavonoids are well known parts of polyphenols are present in various plants and possess many actions e.g. antioxidant, anti-mutagenic, anti-inflammatory, antiviral, anti-allergic and anti-neoplastic (Khan et al., 2012). The higher flavonoids contents were found in *Myrsine africana* leaves methanol extract 90±3.4 mg/g dry weight which is slightly higher than other extracts tested such as chloroform extract of *Myrsine africana* leaves 86±2.8 and methanol and chloroform extract of *Myrsine Africana* fruit 88±2 and 80±1.5.

Most of compounds which have been found in plant extracts have potent antioxidant properties. Plants which contain antioxidants play a vital role for human health. They protect organisms by oxidative damage and prevent various diseases such as cancer, heart diseases, neurological diseases and diabetes (Shariatifar *et al*., 2014). The DPPH scavenging assay is used to evaluate the sample ability as hydrogen atoms donor while changing DPPH radical into DPPH-H (reduced form). In the FRAP, Ferric reduces to ferrous ion due to the presence of antioxidants. FRAP deals sample antioxidants as reductants during redox associated colorimetric reaction (Prasad *et al*., 2010).

Table 1. IC_{50} Values of various plant extracts of Medicinal plant extracts

	ABTS radical cation decolorisation Assay	Reducing power Assay	DPPH free radical scavenging Assay	Iron chelating Assay	Hydrogen peroxide ASSAY	Hydroxyl radical scavenging Assay	Superoxide Assay
Myrsine Fruit M	85±9.1	344.6±7	58.6±6.1	35.9±2	14.7±2	26±2.2	131.3±1.7
Myrsine Fruit C	98±5.6	260±4.1	69±2	50±7	25±3	38±6.4	148±4.4
Myrsine Leaves M	119±7.9	25.7±2	15.7±3	29.2±.7	16.8±2.1	14.5±0.84	116.6±2.8

Myrsine Leaves C	160.2±1.7	220.5±11	30.5±1.6	38±4	18.8±4	32.5±0.8	125.6±6.2
Gallic acid	229±15	39.2±1	24.7±2	34.8±2	13.1±1	16.2±1	134.2±5.6

C =chloroform and M = methanol whereas significance level is <0.05

IC_{50}value is the half inhibitory concentration that measures the substance of effectiveness while inhibiting a particular reaction. According to results, highest IC 50 values of *Myrsine africana* leaves were found to be against Hydroxyl radical, Hydrogen peroxide and DDPH assay (14.5±0.84, 16.8±2.1, and 15.7±3 µg/ml) and indicate good antioxidant potential as compared to all other extracts (Table. 1). Higher antioxidant activities of plant extracts could be attributed with higher level of flavonoids and phenols obtained from fruit and leaves extracts. Results obtained in current study are comparable with results of antioxidant potential of plant extracts reported in literature by other authors including (Aberoumand and Deokule., 2008).

Most of the antioxidants methods used for antioxidants assays showed strong correlations with phenolic contents. The strong relationship with phenols and antioxidants method revealed that the antoxidant activity is frequently contributed by phenols (Dudonne *et al*., 2009). IC50 of plants were significantly correlated with total phenolic contents and total flavonoid contents. Secondary metabolites such as flavonoids are the diverse group of natural compounds and are important antioxidants, while the phenols consists of the major compounds that imposed the properties of antioxidants as well as free radical scavenging activities. Flavonoids also impart pharmacological and biochemical effects such as hepato-protective, anti-cancerous and antioxidant activities (Hussein *et al*., 2012).

The Infectious diseases are the reason of mortality in humans and to control such infections pharmaceutical industries has been manufacturing different antibacterial drugs. Those antibiotics are becoming ineffective as resisted by the bacteria. Many plants are called medicinal as they have substances those are curing human diseases. Medicinal plants knowledge is occasionally known as curative reserve for local communities and pays occasionally to the health care (Betoni *et al*., 2006). Extracts of seven different plants were analyzed against different bacterial strains and tested plants showed good activity against *Staphylococcus aureus* and *Escherichia coli*. Plants extracts

were assessed against different gram positive and negative bacterial strains. Different fractions of plants were evaluated and results are in Table 2.

The plant extracts exhibited significant activities against different bacterial strains in different solvents. These plants extracts were active against different diseases and this led to the discovery of novel compounds. *Myrsine africana* leaves methanol was found to have higher activity against *E. Coli* 25±1.5mm zone followed by chloroform extract 22±1.56mm zone against *S.aureus*. *Myrsine africana* fruit methanol extract showed highest activity against *S. aureus* 22±6mm zone. Some studies showed that the methanolic and chloroform extracts of the aerial parts of *Myrsine africana* showed good activity against *K. pneumoniae*. Chloroform and aqueous extract of *Myrsine africana* showed no activity against *E.coli* (Ahmad *et al*., 2011). Some bacterial strains, Gram-negative were more resistant as compared to Gram-positive bacteria. Streptomycin was the standard drug used in this assay. Minimum Inhibitory Concentration is the minimum quantity of an antimicrobial substances those stops the growth of bacteria after the incubation period. In diagnostic labs MIC was used to check resistance. It is commonly used as an instrument in the latest vitro studies for the research purpose (Andrews, 2001). Minimum inhibitory activities of plant extracts was assessed against two strains of bacteria and results obtained indicates lower level of MIC values for various plant extracts indicating their suitability as antimicrobial drugs.

Table 2. Minimum Inhibitory Concentration of Medicinal plants

Extracts	*Staphylococcus aureus*	*Echerichia Coli*
Myrsine africana Leaves Methanol	6.25mg/ml	12.5mg/ml
Myrsine africana Leaves Chlorofom	3.125mg/ml	3.125mg/ml
Myrsine africana Fruits Chloroform	6.25mg/ml	12.5mg/ml
Myrsine africana Fruits Methanol	3.125mg/ml	3.125mg/ml

Myrsine africana leaves showed higher results against all strains as compared to *Myrsine africana* fruits. *Myrsine africana* has multiple roles starting from the flavoring agent, anti- rheumatic and tuberculosis effect to the inhibitory and larvacidal activities and possesses diversity of compounds such as benzoquinones, triterpenes, steroids, flavonoids, flavonol glycosides, Myrsinone A and B (Ahmad *et al*., 2011). Rifampicin was the standard drug showed minimum inhibitory concentration at 0.125 against H37Rv strain. Percentage inhibition was measured and results were given in the

Table 3. Minimum inhibitory concentrations of above three strains were tested against plants extracts and showed great resistant to tuberculosis strain for their growth given in the Table 4.

Table 3. Anti-tuberculosis activity of Medicinal plants

Extracts	**Isolates**	**Mean CFU on media**				**Percentage Inhibition**		
***M. africana* Leaves**		Control	5mg/mL	10mg/mL	50mg/Ml	5mg/mL	10mg/mL	50mg/mL
	bg 1972	130	71	40	0	46	69	100
	H37Rv	140	20	0	0	86	100	100
	bg 206	150	42	15	0	73	90	100
***M. africana* Fruit**	bg 1972	130	65	50	0	50	62	100
	H37Rv	140	35	0	0	79	100	100
	bg 206	150	45	20	0	70	87	100

Percentage Inhibition = Cc – Ct/Cc x100 Cc = No of colony in the control media slope, Ct = No colony in the Test media slope

Table 4. Minimum inhibitory concentration of Medicinal plants

Plant names	**Strains**		
	H37Rv	bg 1972	bg 206
ML	6.25	25	12.5
MF	12.5	25	12.5
Rif	0.125	0.5	0.25

Whereas ML =*Myrsine africana* Leaves, MF = *Myrsine africana* fruit and Rif = rifampicin.

The plants extract tested in this study show stronger resistance against all tested strains of Mycobacterium tuberculosis and this might be due to presence of bioactive components in plant extracts with the methanol that are possibly anti-mycobacterium metabolites. Tuberculosis is responsible for many fatalities in the world and TB patients need to have extensive chemical treatment and ultimately it causes adverse effects to patient health. To reduce the uses of synthetic drugs resistance medicinal plants provide a great confidence as a potential source of bioactive anti-mycobacterium metabolites (Gemechu et al., 2013).

Hemolysis was induced in RBCs by H_2O_2 that is a toxicant and has oxidizing nature lead to degradation of cell membrane and finally hemoglobin discharged from cell. H_2O_2 arouse OH radical's production with Fe2+ mobilization by Ca2+ through Fenton reduction reaction. The above factors totally cause cell membrane disruption, indicating cell lysis (Kadali *et al*., 2016). Anti-hemolytic activity of Methanol extract of *Myrsine africana* fruit and leaves were found to be (70±2.4%) and (72±1%), while chloroform extract showed of fruits and leaves showed (60±3.8%), and (62±0.5%). It was observed that H_2O_2 is toxicant that causes degradation in cell and also produces reactive oxidants. The above antioxidant studies of these plants support the results of anti-hemolytic activity by showing strong effects. This activity was not previously reported in any tested extracts. When red blood cells are exposed to injurious substances e.g. methyl salicylate and phenyl hydrazine causes membrane lysis along with hemoglobin's hemolysis and oxidation. The effects of hemolysis in hypotonic solution are associated with excessive fluid accumulation in cell resulting in membrane rupturing. RBC membrane injury makes the cell susceptible to secondary damage by free radical produced by lipid per-oxidation. This is frequently happening and causes bio-molecules breakdown leads to free radicals formation which increase cellular damage. Free radicals e.g. lipid peroxide and superoxide are reportedly produced in conditions like stress hemolysis is due to destabilization of cell membrane (Durairaj et al., 2014).

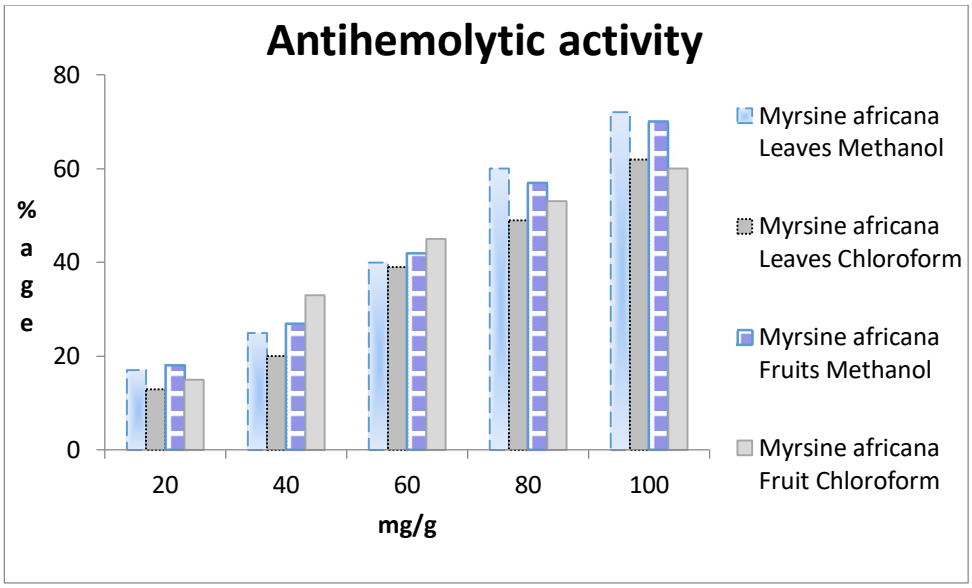

Figure 1. Anti-hemolytic activity of different concentrations of *Myrsine africana*

Plants extracts were analyzed against tumor which was induced by *Agrobacterium tumefacien* with different fractions. Plant extracts shows good defense as shown in Table 33.Potato disc assay of these plants extracts were assessed with different fractions. 10, 100 and 1000µg/ml concentrations were analyzed in the four fractions (Methanol, Ethanol, chloroform and n-hexane). Chloroform and ethanol extracts of *Myrsine africana* leaves showed 88±9.5% and86.5±5% antitumor activity. They were found to be active in both polar and non polar solvents it can be due to the type of phyto-constituents which are intended to be dissolved in particular solvent, nature of solvent and extraction procedure. *Myrsine africana* fruit showed high antitumor activity in non-polar solvents Chloroform (83.4±7.1%) and n-hexane(82.4±7%).It could be due to less polar or non polar compounds present in these plants extracts and found to be active not only against agrobacterium tumefacien strains but also against bacteria's, cancer cells and tuberculosis strains which justifies the significance of current results. As the concentration increases ultimately activity also increases. This study is relatively a quick, reasonable, nontoxic and statically consistent method for the selection of murine leukemia (antitumor activity) (Hussain *et al*., 2007).The obtained values were significantly different $p<0.05$.

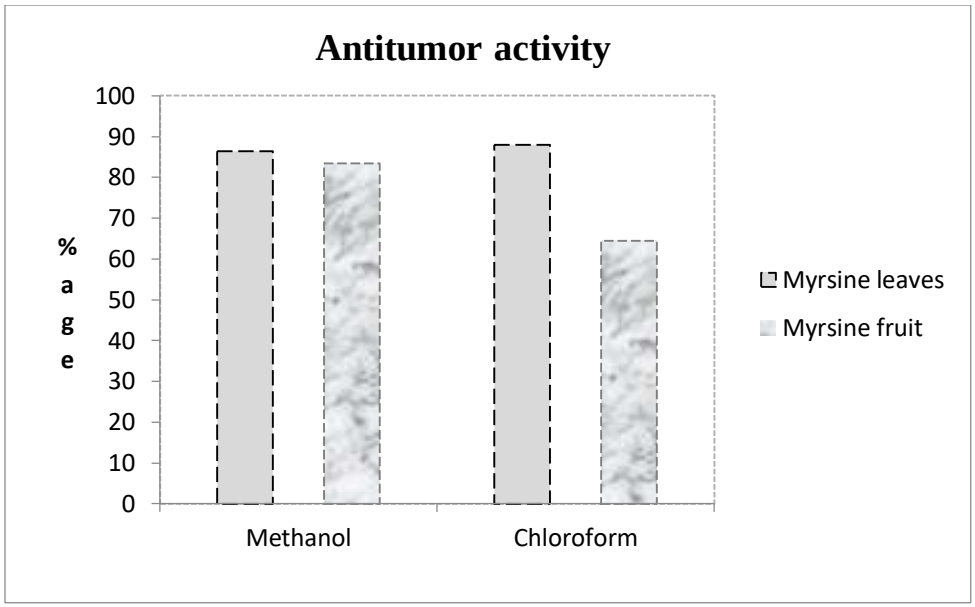

Fig.2. Antitumor activity of *Myrsine africana* Leaves and Fruit

CONCLUSIONS

Drugs discovery from medicinal plants still remains an important area of human health. The possible benefits of plant based medicines have led to unscientific exploitation of the natural resources, a trend that is being observed globally. Therefore drug discovery from natural source involves a complicated approach combining botanical, phytochemical, biological and molecular techniques. The findings revealed that these plants possessed biologically active secondary metabolites can be further isolated in future pharmacological studies. These plants need to examine more to obtain new compounds, might be useful for preparation of new drugs required to control various diseases of human and animals.

REFERENCES

Ali A , Deokule SS (2008). Comparison of phenolic compounds of some edible plants of Iran and India. Pak J Nutr. 7(4): 582-585.

Adeolu A A, Florence O J, Anthony J A, Patrick J M. (2009). Antioxidant properties of the methanol extracts of the leaves and stems of Celtis africana. Rec Nat Prod. *3*(1): 23.

Bashir A, Sadiq A, Shumaila B, Farrukh H, Muhammad I C. (2011). Insecticidal, brine shrimp cytotoxicity, antifungal and nitric oxide free radical scavenging activities of the aerial parts of Myrsine africana L. Afr J Biotechnol. 10(8): 1448-1453.

Olayinka A O & Anthony O I. (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Compl Alternative Med. 10(1):21.

Heshmatollah A, Razieh A, Mahboobeh Z, Mohammad A E, Shahram E, Seyed F N, Seyed M N. (2013). Antioxidant and antihemolytic activities of ethanolic extract of flowers, leaves, and stems of Hyssopus officinalis L. Var. angustifolius. Int J Food Prop. 16(5):1169-1178.

Jennifer A M. (2001). Determination of minimum inhibitory concentrations. J antimicrob Chemother. 48 (suppl 1): 5-16.

Ashafa AOT, Grierson, DS , Afolayan AK. (2010). In vitro antioxidant activity of extracts from the leaves of Felicia muricata thunb. an underutilized medicinal plant in the eastern cape province, south africa. Afr J Tradit Complement Altern Med. *7*(4).

Charles B , Irwin F. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 44(1):276-287.

Joyce E C B, Rebeca P M, Lidiane N B, Luiz C D S, Ary F Junior. (2006). Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem Inst Oswaldo Cruz. 101(4): 387-390.

Ikram D, Marie-Laure F, Marianne S, Fatima B, Asmae A, Mohamed B et al. (2017). Chemical composition, vasorelaxant, antioxidant and antiplatelet effects of essential oil of Artemisia campestris L. from Oriental Morocco. BMC compl alternative med. 17(1):82.

Teresa C P D, Vitor M C M., & Leonor M A. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem Biophys. 315(1): 161-169.

Stephanie D, Xavier V, Philippe C, Marion W, Jean-Michel M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J AGR FOOD CHEM. 57(5) :1768-1774.

Varalaskshmi D, Muddasarul H, Garima S, Sankar P P B, Rukkumani R. (2014). Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. Asian Pac J Trop Med. 7: S398-S404.

Ette E , Paul N. (2009). In vitro antimicrobial activities of extracts of Carpolobia lutea root. Pak J Pharm Sci. 22(3).

Abdella G, Mirutsey G, Adane W , Gobena Ameni. (2013). In vitro Anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis Strains. BMC Compl Alternative Med. 13(1): 291.

Mahmood G, Mohamad Sherafati, Gholam A P. (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (Capsicum annum) fruits at two different harvest times. J Funct Foods. *3*(1): 44-49.

Hernandez-Hernandez AB , Alarcon-Aguilar FJ, Almanza-Perez JC, O Nieto-Yañez O, Olivares-Sanchez JM , Duran-Diaz A, Canales-Martinez. MM (2017). Antimicrobial and anti-inflammatory activities, wound-healing effectiveness and chemical characterization of the latex of Jatropha neopauciflora Pax. J Ethnopharmacol. 204: 1-7.

Afzal H, Abdus S, S K Singh, M N Ahsan, M W Haque, A Faruk, & F J Ahmed. (2016). Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. Drug delivery, 23(2), 642-657.

Hidayat H, Karsten K, Zia Ullah, Siegfried D, Barbara S. (2007). Bioactive chemical constituents of two endophytic fungi. Biochem Syst Ecol. 35(12): 898-900.

Mohsen H, Carola F V E, Andrej C, Dejan D , Freddie H F. (2012). Prospective randomized clinical evaluation of conventional single-bundle, anatomic single-bundle, and anatomic double-bundle anterior cruciate ligament reconstruction: 281 cases with 3-to 5-year follow-up. Am J Sports Med. 40(3): 512-520.

Raissa B I, Maicon M L, Roberto M K, Luis F M, Flora M F M, Claudia A L C & Roberta G C et al. (2017). Anti-inflammatory, antimycobacterial and genotoxic evaluation of Doliocarpus dentatus. J. Ethnopharmacol. 204: 18-25.

Venkata N K, Tadi Ramesh, Sudhakara R P & B V Sandeep. (2016). Phytochemical analysis and antibacterial activity of Crossandra infundibuliformis Ethanolic stem extract. Phytochemical analysis. 1(1).

Fatma P K, Arzu U T, Alper K, Vakhtang M, Andre P, Jean L. (2017). In vitro cytotoxic, antibacterial, anti-inflammatory and antioxidant activities and phenolic content in wild-grown flowers of common daisy—A medicinal plant. J Herb Med 8: 31-39.

Rahmat A Khan, Muhammad R K, Sumaira S, Mushtaq A. (2012). Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill. Chem Cent J 6(1):12.

Wanying L, Hang Y, David B, Jianbo X, Ying Z , Ruibing W. (2017). Supramolecular formulation of nitidine chloride can alleviate its hepatotoxicity and improve its anticancer activity. *Food Chem Toxicol*.

Joon-Kwan M , Takayuki S. (2009). Antioxidant assays for plant and food components. J. Agric. Food Chem. 57(5): 1655-1666.

Seyed F N, Nady B, llkay E O, Arash B, M Daglia, Seyed M N. (2016). Rhodiola rosea L. and Alzheimer's disease: from farm to pharmacy. Phytother Res.30(4):532-9.

Takeshi N, Takao M, Toshio N. (2005). Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) Equisetum arvense L. Food chem. 91(3): 389-394.

Nagendra K P, Bao Y, John S, Chunyan Y, Mouming Z, Sophia X, Yueming J. (2010). Enhanced antioxidant and antityrosinase activities of longan fruit pericarp by ultra-high-pressure-assisted extraction. Anal. J Pharmaceut Biomed. 51(2): 471-477.

Ramamurthy CH, M Padma, I. Daisy M S, R Mareeswaran, A Suyavaran, M. Suresh K, K Premkumar, C Thirunavukkarasu. (2013). The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. Colloids Surf. B: Biointerfaces. 102: 808-815.

Azadeh S, Mahdieh R, Mona E & Mahsa H J. (2014). Effects of Nigella sativa L. seed extract on fatigue, blood biochemical parameters and thyroid function in male mice. Chin Med J. 5(1). DOI: 10.4236/cm.2014.51003.

Milan S S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. *Kragujevac* J Sci. 33(2011): 63-72.

Kenneth K W T, Xu W, Chun Y, Stella C, Sheng Y, Onat K, Thomas E et al. (2017). Reversal of multidrug resistance by Marsdenia tenacissima and its main active ingredients polyoxypregnanes. J. Ethnopharmacol. 203: 110-119.

Muhammad Y, Bushra S, Poonam S N, Richard O A. (2016). Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC–ESIMS/MS identification of phenolic components. Food chem. 199: 307-313.