ANALYSIS OF CHEMICAL COMPOUNDS FROM LEAVES EXTRACTS OF *MYRSINE AFRICANA* L. AND ASSESSMENT OF BIOACTIVITIES OF VARIOUS EXTRACTS

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ABSTRACT

The pharmaceutical industry relies on natural compounds derived from plants as a primary raw resource in the creation of new medications.

The North Eastern part of Pakistan is home to the forest regions where the popularly ingested medicinal herb, Myrsine africana L., flourishes. This research synthesized, chemically analyzed, and evaluated bioactivities of different leaf extracts. A greater amount of tannins, total flavonoids, and total phenols were extracted from fruits using methanol. The HPLC results showed that quercetin was present at a greater concentration than rutin and p-coumaric acid. In contrast, GC-MS analysis revealed a statistically significant concentration of 10 different fatty acids, both saturated and unsaturated. Research conducted in a controlled laboratory setting revealed that leaf extracts had a reduced cytotoxic activity, greater antioxidant values, and a larger zone of inhibition against several bacteria, including S. aureus, E. coli, K. pneumonia, B. subtilis, and Mycobacterium TB. Extraction of M. africana leaves yielded chemical components that were consistent with its traditional medical uses.

Keywords: *Myrsine africana,* chromatography, extraction, bioactivities assessment

INTRODUCTION

Researchers are always looking for novel ways to use the many natural compounds found in plants to create pharmaceuticals (Carvalho and Barata et al., 2017). Traditional medicine relied heavily on all-natural remedies for a wide range of medical conditions. The bioactive chemicals found in natural goods are many. According to Reiz et al. (2012), these bioactive molecules have the ability to combat many substances that cause illness. Diverse groups of researchers have isolated secondary metabolites from plants, and these compounds range widely in structure and pharmacological activity (Gasecka et al., 2017). According to Zhao et al. (2009), traditional medicine is relied upon by over 85% of the global population when it comes to treating a range of human disorders.

Natively known as Khokhal, Myrsine africana L. (Myrsinaceae) is a plant family member. Lall et al. (2017) and Gul et al. (2017) state that this plant is often found in the drier tropical and subtropical regions of Asia and Africa. It prefers an annual rainfall of 600–800 mm and can withstand temperatures ranging from 22 to 35 ºC. It may be found at elevations of up to 3,800 m. The northeastern region of Pakistan experiences this often throughout the winter and summer rainy seasons. This plant may reach a height of 2 meters and is often seen growing on rocky forest slopes and evergreen shrubs. In addition to their traditional uses as a cologne, carminative, taster, and spice mediator, the fruits are edible. Many human aliments, including blood decontaminant, toothache, bronchitis, cough, TB, and many more microbiological disorders, are treated using extracts from the fruit and leaves. Earlier, Ahmad et al. (2016) reported the antibacterial benefits of M. africana leaf and fruit. which were investigated by Kishore et al. (2018), as well as the separation of flavonoids and flavonoid glycosides. Myrsigenin is an antispasmodic compound derived from a methanolic extract of Myrsium africana (Azam et al., 2011). Eighty percent of the world's population relies on medicinal substances derived from plants, according to WHO data, and many of the pharmaceuticals available today are phytochemical preparations (Ekor, 2014). Digitalis, ergotamine, quinine, salicylates, and others fall under this category of medicinal medications.Research by Mustafa et al. (2019) and Khadam et al. (2019).

Therefore, in order to keep their production going, it is necessary to encourage conservation of these plants that are in risk of extinction.

MATERIALS AND METHODS

Collecting Myrsine africana leaves began in the Murree districts of Pakistan's District Rawalpindi. Although this region receives less precipitation each year than the rest of the nation, it is nonetheless classified as a dry zone. An expert taxonomist verified the identity of the plant samples and returned voucher specimen (No.235) to the relevant department. After being sun-and shadow-dried, the plant components were oven-dried and then crushed into a powder. Using a rotary evaporator and the technique of Soxhlet apparatus, 100 g of the sample was macerated with distilled water, methanol, ethanol, chloroform, and n-hexane.

Analysis

Following the methodology outlined by Abbasi et al. (2015), the total flavonoids, phenols, and tannins were determined using a modified Folin-Ciocalteu colorimetric approach.

HPLC Analysis

The HPLC study used a Shimadzu HPLC system from Tokyo, Japan, which came with a UV/visible detector and a C18 column measuring 25mm × 4.5mm and 5µm in particle size. A 36:64 ratio of acetonitrile to 0.1% phosphoric acid was used to elute the components. Twenty microliters was the volume of injection for every sample. We used a flow rate of 1 ml/min and wavelengths of 280 nm and 285 nm to analyze the flavonoids. All assays were run in triplicate, with quercetin serving as the standard.

GC-MS analysis

The following parameters were used in the GC-MS study by Shimadzu: a capillary column RTx-5MS measuring 30m x 0.25mm x 0.25µm, a split injection operating at 250ºC, helium as the carrier gas, and a column flow rate of 1.2mL/min in a

constant linear velocity mode. From 4 °C each minute up to 150 °C, it is the temperature schedule for the column oven. At 275ºC, the splitting share fraction was 50:1, the injection volume was 0.2µl, and the carrier gas employed was N2 (1.0mL/min). We checked the derivatized fruit extract with the spectrum data base of "The National Institute of Standards and Technology" (NIST) to identify the chemicals. The percentage for each chemical was calculated by comparing its peak area to the overall peak area.

The Antioxidants Activity

Brand-Williams et al. (1995) described a method for measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity. Using the procedure described by (Mensor et al., 2001), the percentage of scavenging activity was calculated by reacting the reaction mixture with the stable DPPH molecule in an ethanol solution. (Ashafa et al., 2010) outlined the steps to take in order to conduct the 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) test. Various tests were conducted to measure superoxide radical, hydroxyl radical, and iron chelating, as outlined in (Cefarelli et al., 2006; Dehghan and Khoshkam 2012). The (IC50 µg/ml) was determined from the dose response curve after 50% inhibition.

The antibacterial activity

Four bacterial strains—Staphylococcus aureus (ATCC 6538), Klebsiella pneumonia (MTCC618), Escherichia coli (ATCC 15224), and Bacillus subtilis (ATCC 6633)—were tested for antibacterial potential using the agar well diffusion technique with M. Africana leaf extracts. The clinical and pharmacological significance of these bacterial strains was considered when their selection was made (McCracken and Cowsan, 1983). Oxygen detection was performed at 420 nm using a UV/visible spectrophotometer and the standard antibiotic medication Cefixime. The minimum inhibitory concentration (MIC) of the extracts was determined to be the concentration at which bacterial growth was inhibited after 24 hours of incubation (Upadhyay, 2015).

Test for the Anti-Mycobacterium tuberculosis

This experiment included a sensitive strain H37Rv of Mycobacterium TB in addition to drug-resistant strains bg 206 and bg 1972. All strain inoculums were created.

About 60µl was streaked onto Lowenstein Jensen (LJ) medium slants using a loop with an exterior diameter of 3mm from each strain of Mycobacterium TB suspensions that had been diluted 10-2. The fruit extracts were added to the medium at three different concentrations: 05 mg/mL, 10 mg/mL, and 50 mg/mL. The stated approach was used to carry out the anti-mycobacterial activity of the leaf extracts (Martins et al., 2014).

STATISTICAL ANALYSIS

The data obtained during various analyses were analyze statistically for various parameters.

RESULTS AND DISCUSSION

Table 1 shows that among the extracted phytochemicals from the different leaves of M. africana, the methanolic fruit extracts had the highest concentrations of tannins, total phenols, and flavonoids. Consumers may get the health advantages of phytochemicals, which are naturally occurring chemical compounds generated by plants (Baskaran et al., 2011). Fruits and vegetables include phytochemicals that may protect the body against cancer and oxidative stress, among other disorders (Abbasi et al., 2015; Ujowundu et al., 2008).

Table 1: Phytochemicals analysis from leaves extracts of *M. africana*

Extracts	Total flavonoids mg GA/100g	Total phenol mg GA/1oog	Tannins mg GA/100g
Methanol	17.16 ± 2.11Þ	24.36 ± 2.25ª	7.16±0.38Þ
Ethanol	16.17± 1.36ª	12.73 ± 1.54ª	5.16±0.52ª
Chloroform	13.34 ± 0.27Þ	11.26 ± 1.7Þ	4.53±0.31ª
Aqueous	7.08 ±0.54	3.19± 0.68	0.71±0.45

Mean ± SD (n=3), ª = p<0.01,Þ= p<0.05.

HPLC analysis

According to the results of the HPLC analysis, the methanolic extracts of the leaves included quercetin, rutin, and p-coumaric acid (As shown in Figure 1). According to the increased flavonoid content of the fruit extract, the leaves of M. Africana have the potential to be used in the treatment of local ailments. After analyzing a variety of plant extracts, Movileanu et al. (2000) also obtained findings that were comparable to those described above.

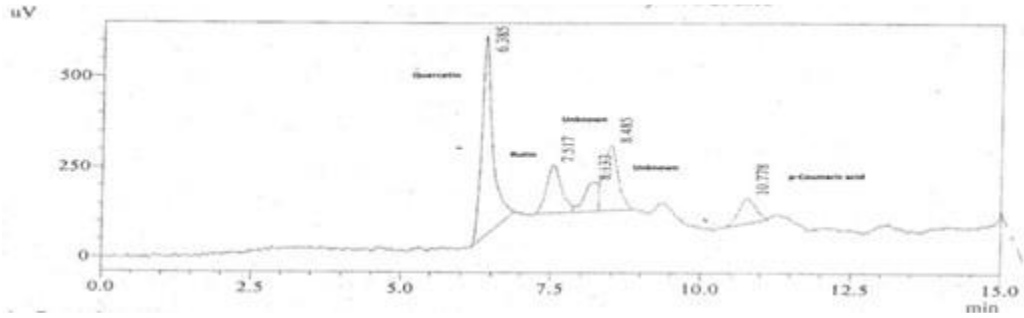

Figure 1. The HPLC profile of flavonoids from methnolic leaves extract of M. Africana quercetin was used as standard

GC MS analysis

The findings of the GC-MS analysis of M. Africana leaf extracts in methanol are shown in Fig. 2

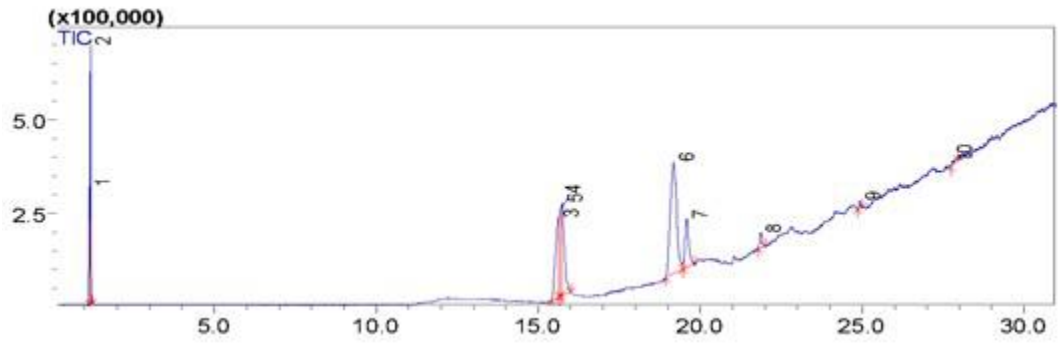

Figure 2. GC-MS profile of constituents of methanolic leaves extract of *M. Africana*

There were a total of ten chemical compounds found in the leaf extracts using GC-MS analysis (Fig. 2), with seven of those compounds taking center stage. The compounds' peak regions were determined by comparing the GC-MS chromatograms, and the necessary information was retrieved from the NIST library database. With a peak of 6.07%, hexacosanol is indicated. C27H56 refers to Two peaks: one containing ethanol (25.95%) and one containing isobutylalcohol (1.73%). Peak 4 of C4H10O corresponds to ascorbic acid, peak 5 to octadecenoic acid, peak 6 to stearic acid, peak 7 to palmitoylchloride, peak 8 to hexacontane, peak 9 to phosphoheptacos, and peak 10 to C18H34O2. Peak 10 heptacosylhepta fluorobutyrate (3.60%), and C44H84O8 generally. The formula is C31 H55F7O2. Based on a thorough review of the existing literature, it has been determined that the compounds on peaks 8, 9, and 10 have not been previously reported in plant extracts, particularly from M. africana or related plant species. Consequently, this study represents the first investigation into these compounds from M. africana leaves extracts. Other authors have also reported similar results from other plant extracts (Mani and Murugan 2017). Chemical components, GC-MS retention time (R.T.), and peak area (%).

Table 3: Antioxidant effects of leaves extracts of *M. Africana* (IC50 values µg/ml)

Extract 100 µg/ml	DPPH	H_2O_2	ABTS	Reducing power	Superoxide	Iron chelation
Ethanol	38.16±0.2[α]	43.51±0.1a[α]	46.72±3.1[α]	64.53± 2.6[α]	106.54± 1.5 Þ	32.32± 1.5[α]
Methanol	17.23±0.4 Þ	41.25±2.5[α]	39.42±2.3[α]	61.18± 3.5 Þ	101.23± 1.5 Þ	23.64± 2.5 Þ
Chloroform	25.32±0.4 Þ	43.62±2.1[α]	41.21±1.7[α]	72.44± 2.6 Þ	105.04± 1.2Þ	26.07± 3.1 Þ
Aqueous	41.13±0.3[α]	52.43±3.5 Þ	48.51±3.8[α]	92.16± 3.4[α]	114.17± 1.3[α]	41.32± 1.2[α]
Ascorbic acid	8.24±0.81[α]	7.52 ±0.9[α]	6.34±0.35 Þ	11.35 ±1.1 Þ	31.25± 2.8[α]	34.06± 1.8[α]
Gallic acid	4.81±0.3 Þ	1.68±0.3 Þ	1.23±0.34 Þ	2.35±0.3 Þ	17.26±1.6 Þ	20.18±1.8 Þ

Means ± SD, (n = 3), whereas [α] = $p<0.01$,Þ= $p<0.05$. Gallic acid was used as standard

Table 4: Antibacterial activities of various leaves extracts; zone of inhibition in mm

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime
Ethanol	19.6±0.6	18.4±0.9	19.5±0.3	22.6±0.4	16.5±0.9	12.5±0.3
Methanol	23 .5±0.7	22.5±0.7	22.9±0.5	21.7±0.3	13.6±0.9	11.5±0.3
n-hexane	18.3 ±0.6	18.7±0.5	18.8±0.4	17.5±0.8	13.8±0.5	10.8±0.5
Chloroform	19.8±0.5	18.7±0.6	15.4±0.6	16.6±0.7	14.5±0.5	11.8±0.7
Aqueous	11.5±0.6	13.5±0.8	12.7±0.8	12.5±0.6	11.6±0.5	10.6±0.7

Results mean± S D, (n=3).

Table 5: MIC values minimum inhibitory concentration (µg/ml) for various bacterial strains against leaves extracts

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime
Ethanol	0.7±0.3	1.5±0.4	0.8±0.3	1.6±0.5	1.3±0.7	1.5±0.5

Methanol	0.3±0.1	2.3±0.4	0.7±0.3	1.3±0.4	1.4±0.5	1.1±0.7
n-hexane	1.5 ±0.3	1.6±0.8	0.9±0.5	1.4±0.8	1.5 ±0.4	1.3±0.6
Chloroform	1.8±0.6	1.7±0.5	1.4.±0.5	1.5±0.7	1.5±0.3	1.3±0.8
Aqueous	1.2±0.6	1.6±0.7	1.6±0.4	1.8±0.5	1.5±0.4	1.5±0.5

Results are Means ± SD, (n = 3)

Table 6: Anti-tuberculosis activities of leaves extracts on LJ Media

Extracts	Isolates	Mean CFU on media				Percentage Inhibition		
Methanolic fruit extracts	H37Rv	140	80	76	20	43	46	87
	bg 206	150	90	60	35	40	60	76
	bg 1972	130	86	64	40	34	51	68

Cc / Ct = No of colony / No of colony in the control media slope Inhibition (Cc-Ct/Ccx 100).

Antioxidant assays

It was determined if M. Africana leaf extracts had any antioxidant effects. According to table 3, the methanol leaves extract showed stronger antioxidant activities, although its free radical scavenging activity was reduced in the DPPH test (IC50 18.23±0.45µg/ml) compared to the other assays.

Antibacterial activities of leaves extracts

In comparison to conventional antibiotics, methanolic leaf extracts were tested for their ability to suppress the development of several bacterial strains, including S. aureus, E. coli, K. pneumonia, and B. subtilis (tables 4 and 5). The fruit extracts had significant antibacterial activity against both Gram-positive and Gram-negative bacteria, indicating that the leaves of M. africana contain natural chemicals with antimicrobial properties, such as flavonoids, which might be used to combat infectious disorders affecting humans. Hence, the current study backs up the previous data given by other writers, although with minor adjustments (Fawole et al., 2008).

The widespread development of antibiotic resistance poses a serious threat to public health. Traditional medicine has a long history of use as a treatment for infectious diseases, and there is promising evidence that plants with medicinal properties might help combat the spread of antibiotic-resistant diseases (Shah et al., 2019; Muniandy et al., 2019; Baskaran et al., 2011).

The high antimicrobial activity of fruit extracts is shown by their low inhibitory concentration. The inhibitory concentrations of each fruit solution were found to be variable, as shown by the MIC values of different extracts. It is possible that the fruit components' inherent purity in these solvents contributed to the decreased MIC level seen in the methanolic leaf extracts (table 5). Thus, the results of this study's analysis of the antibacterial capacity of leaf extracts were consistent with those of other authors (Guessan et al. 2007).

Anti-Mycobacterium tuberculosis assays

The effectiveness of three leaf extract concentrations against H3Rv, bg206, and bg1972 strains of Mycobacterium TB were recorded in table 6. According to McCracken and Cowsan (1983), after eight weeks of incubation, the number of colony forming units (CFU) was ultimately observed for each slope. To determine the percentage inhibition, we compared the extract-free slope of each strain to the colony count on plates containing extracts. The fruit of the African mango tree has potential as a remedy for a number of chest-related ailments, including TB, according to research on extracts from the fruit's leaves.

Plant extracts are being used as both food and medication due to the presence of biologically active components. Isolation, representation, and in-vivo and in-vitro evaluation of biological activities of these phytochemicals are gaining increasing attention because of their enormous therapeutic potential, either alone or in combination, for the treatment of a wide range of human illnesses. The results show that M. africana fruit extracts include a wide range of chemical components that have anti-tuberculosis, anti-proliferative, antioxidant, and cytotoxic effects. The HPLC analysis revealed that the main components detected in the M. africana leaf extracts were p-coumaric acid, rutin, and quercetin. Which, together with fatty acids and other organic molecules identified by GC-MS, are expected to serve as the primary antioxidants. According to Cefarelli et al. (2006) and Ujowundu et al. (2008), flavonoids have a number of useful biochemical features, including the capacity to protect cells from oxidative stress. The research found that M. Africana leaf extracts had beneficial effects against the tested strains of bacteria and mycobacterium at higher concentrations. Zone of inhibition evaluations were greater than those of other writers (Baskaran et al., 2011). Consumption of flavonoid-rich fruits and vegetables has been linked to a decreased risk of cancer in prior research (Park et al., 2008; Ferguson et al., 2004). Thus, flavonoids have protective effects against

free radicals, germs, viruses, and inflammation. In addition to bolstering the body's antioxidant defenses, flavonoids also activate the system of defensive enzymes. By protecting against cancer, heart disease, respiratory illness, arthritis, and the effects of aging, flavonoids play an important role in modern medicine. The polyphenolic structure of flavonoids often renders them well-suited for a variety of pharmacological actions. Antioxidants in flavonoids work by scavenging free radicals or chelating melting ions; this prevents free radical damage to human cells, which in turn prevents major illnesses in humans (Ekor, 2014).

CONCLUSION

Find out what the leaf extracts' chemical make-up and biological activity are. The analysis of M. africana leaves revealed the presence of beneficial chemical components. This practical plant should be protected because of its widespread usage in traditional medicine.

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