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Research Article

Preliminary Phytochemical, Antioxidant and Antimicrobial Investigation of Selected Medicinal Plants of Khyber Pakhtunkhwa

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Abstract: The majority of the people living in rural areas are still relying on traditional medicine for their primary healthcare needs. The locally available medicinal plants contain bioactive compounds that are widely used for the treatment of various chronic diseases caused by multidrug-resistant (MDR) microbes. However, research in this area has been limited. In this regard, the current study was designed on assessing the antimicrobial properties and phytochemical constituents of ethanol, methanol, hexane, ethyl acetate, and aqueous extracts of *Ruellia tuberosa, Aesculus indica,* and *Myrsine africana* against MDR microbes. The results of phytochemical analysis revealed the presence of different classes of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and reducing sugar, though anthraquinons, phlobatanins, and glycosides were absent in the selected plants. Furthermore, the quantitative analysis of methanol extract showed that *M. africana* had notable concentrations of total phenol (3.75 ± 0.05 mg $100g^{-1}$) and total flavonoid (11.39 ± 1.72 mg $100g^{-1}$) contents, while *R. tuberosa* had the highest (0.74 ± 0.06 mg $100g^{-1}$) tannins content. In addition, the lowest concentrations of total phenol (2.88 ± 0.04 mg $100g^{-1}$) and flavonoid (6.77 ± 1.02 mg $100g^{-1}$) were examined in *A. indica*, while the lowest tannin (0.31 ± 0.03 mg 100^{-1}) moiety was observed in *M. africana*. Moreover, the mean highest (46.64%) antioxidant activity was observed for *A. indica*, while the lowest value was observed for *M. africana* (32.56%). The antimicrobial susceptibility test showed that ethanol, hexane, ethyl acetate, and aqueous extracts of the selected plants had remarkable inhibitory potential against the test bacterial and fungal species. The antimicrobial activity of selected plant extracts had no to low and moderate to good inhibitory potential against the test microorganisms.

Keywords: Phytochemical, Total Phenol, Total Flavonoid, Antioxidant, Antimicrobial.

1. INTRODUCTION

Plants serve as a major source of therapeutic compounds that play a crucial role in traditional medicine recipes in remote regions of Pakistan. They provide essential elements for diets, medicines, pharmaceuticals, and food additives, and serve as the basis for various synthetic drugs [1]. Approximately 80% of the global population relies on natural resources for their primary healthcare needs, according to the World Health Organization (WHO). These plants, which have a repertoire of diverse bioactive compounds, are often considered weeds in Pakistan. Similarly, rural populations in India, Iran, Afghanistan, China, and other parts of Asia heavily depend on wild plants for their medicinal needs [2, 3]. Recent research highlights that many commonly used drugs are derived from plants or other natural sources [4]. Plant-derived secondary metabolites are extensively used not only for the treatment of various infectious diseases but also for curing cancer, allergy, inflammation, stress, diabetes, atherogensis, and thrombosis, as well as scavenging free radicals from the body [5, 6]. These pharmacological activities were attributed to the presence of various classes of secondary metabolites that are widely found in different parts of the plants, such as leaves, stems, roots, bark, flowers, fruits, and rhizomes [7]. Phytochemicals, including terpenoids, flavonoids, and carotenoids, contribute to these biological activities [8]. For example, flavonoids, a prominent class of polyphenols extensively found in plants, exhibit a multitude of pharmacological effects [9]. Within

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flavonoids, different subclasses such as flavones, flavonols, flavanones, isoflavones, isoflavans, coumestans, anthocyanins, and pterocarpans exist [10]. Numerous research studies have demonstrated the efficacy of these phytochemicals against various human, livestock, and poultry pathogens within traditional medicinal practices. However, both common and emerging infectious diseases pose significant threats to communities, highlighting the urgent need to identify diverse chemical compounds with improved mechanisms of action against these infections [11].

The free radicals such as hydroxyl, nitric oxide, hydrogen peroxide, and various singlet oxygen molecules are produced by our body during metabolic activities [12, 13]. These free radicals are the major cause of many chronic and degenerative diseases [14]. The oxidative damage caused by free radicals is prevented by antioxidants. The antioxidant compounds are predominantly produced by plants in the form of secondary metabolites such as phenols, vitamins, terpenoids, and flavonoids [15]. Many secondary metabolites produced by plants can also serve as effective drugs against harmful microbes and unwanted cells [16]. Recently, there has been a focus on screening antimicrobial activities due to the rise in antibiotic-resistant infectious agents, leading to therapeutic challenges. Plant-derived antimicrobial compounds have proven highly potent even in small doses, often outperforming synthetic alternatives under similar evaluation methods [17, 18]. The presence of secondary metabolites in plants has long been recognized, serving as inspiration for the development of innovative drug compounds and making significant contributions to human health through natural products derived from plants. Therefore, this study was designed to screen the organic extracts of selected plants for the existence of different classes of secondary metabolites that contribute to antimicrobial and antioxidant activities.

2. MATERIALS AND METHODS

2.1. Plants Collection

In this study, the roots of *R. tuberosa,* nuts of *A. indica*, and aerial parts of *M. africana* were collected from Charsadda, Peshawar, and Swat districts of Khyber Pakhtunkhwa province, Pakistan. The respective plant parts were thoroughly washed with tap water, followed by distilled water, to remove all kinds of adhering materials. The selected part of each plant was stored under shade for complete dryness. The dried plant materials were converted to powder form by using an electrical grinder (Yigan, model WF130). The resulting powdered samples were stored at 4 °C after carefully packing and labeling them in clean Zip-lock bags [19]. The phytochemical and antimicrobial activity was performed at the Department of Agricultural Chemistry, the University of Agriculture Peshawar, and the Pakistan Council of Scientific and Industrial Research Institute (PCSIR), Peshawar, Pakistan.

2.2. Extraction and Fractionation

The extraction was performed by adding ethanol (2.0 l) to the powdered sample (1.50 kg) taken in the separating funnel, employing the cold maceration technique. The crude ethanol extract was collected from the separating funnel and filtered through Whatman filter paper No. 1. The filtrate was then dried at 45 °C using a rotary vacuum evaporator (Heidolph Laborota 4000). Afterward, 100–150 ml of distilled water was added to the dried ethanol extract and then partitioned with hexane and ethyl acetate. The dried hexane and ethyl acetate fractions were obtained by evaporating the respective solvents through a rotary evaporator. The dried ethanol extract and its fractions, i.e., hexane, ethyl acetate, and aqueous, were kept in clean glass vials and stored in the refrigerator at 4 °C for further analysis [20].

2.3. Phytochemical, Antioxidant and Antimicrobial Analysis

The crude ethanol extract and fractions of the selected plants were screened for the presence or absence of different classes of secondary metabolites by the method of Trease and Evans (1989) [21]. Similarly, total phenol and tannin contents were quantitatively estimated using the method of Grubesic *et al.* [22]. Likewise, the total flavonoid content was determined by the method described by Sharma *et al.* [23], while the standard protocol of Akond *et al.* [24] was used for the determination of antioxidant activity. The antimicrobial potential of the extracts was tested against six gram-negative bacteria (i.e., *Klebsiella pneumonia* (ATCC # 13883), *Pseudomonas aeruginosa* (ACCT # 9721), *Escherichia coli* (ACCT # 25922), *Erwinia*

carotovora, Salmonella typhi, and *Agrobacterium tumefacien*), three gram-positive bacteria (*Bacillus subtilis* (ATCC # 6633), *Staphylococcus aureus* (ATCC # 6538), and *Bacillus atropheous*), and one fungal strain, *Candida albicans,* by the disc diffusion method of Bauer *et al.* [25]. The inhibitory zone of the plant extracts was recorded in millimeters (mm) and then expressed in percentages by using the formula: inhibition (%) = sample/control × 100.

2.4. Statistical Analysis

The experiment was laid out in a 2-factorial completely randomized design (CRD) using Statistix 8.1 statistical software. All the data were recorded in triplicate. Mean comparisons were conducted using the least significant difference (LSD) test at a significant level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

The data of phytochemical analysis revealed that crude ethanol, hexane, ethyl acetate, and aqueous fractions of *R. tuberosa, A. indica,* and *M. africana* contain different classes of secondary metabolites such as flavonoids, terpenoids, tannins, and steroids, respectively. Likewise, phlobatnin, glycoside, and anthraquinon were not found in the said plant extracts. In addition, alkaloids were found in the hexane, ethyl acetate, and aqueous extracts of *R. tuberosa* but did not exist in any of the extracts of *A. indica* and *M. africana*. Saponin and reducing sugar were present in all plant extracts except hexane, ethyl acetate, and aqueous fractions of *R. tuberosa* (Table 1). The results of phytochemical screening observed in the course of this study are in close proximity with the earlier reported studies [26-28]. The rationale behind this study lies in the role of initial understanding of phytochemicals in standard herbal drug development processes. This preliminary knowledge of phytochemicals provides insights into the diverse chemical constituents found in plant materials [29]. Moreover, exploring the presence of bioactive agents in plants not only informs but also provides potential precursors for the development of synthetic drugs [30]. The results of total phenol, tannin, and flavonoid content of the selected plants are presented in Table 2. The data showed that *M. africana* had a remarkable concentration of total phenol (3.75 ± 0.05 mg $100g^{-1}$) and total flavonoid (11.39 ± 1.72 mg $100g^{-1}$) contents, while *R. tuberosa* had the highest (0.74 ± 0.06 mg $100g^{-1}$) tannin content. Moreover, the lowest concentrations of total phenol (2.88 ± 0.04 mg $100g^{-1}$) and flavonoid (6.77 ± 1.02 mg $100g^{-1}$) were examined in *A. indica,* while the lowest tannin (0.31 ± 0.03 mg $100g^{-1}$) content was observed in *M. africana*, respectively. ANOVA showed that the values of total phenol, tannin, and total flavonoid contents varied significantly at $p < 0.05$. The results obtained in the course of this study find supportive evidence from previous findings [26, 27, 31-33].

3.2. Antioxidant Activity

The results of the antioxidant activity of selected

Table 1. Bioactive constituents in different extracts of *R. tuberosa, A. indica,* and *M. africana*.

S. no.	Bioactive constituents	Ethanol			Hexane			Ethyl acetate			Aqueous		
		RT	AI	MA	RT	AI	MA	RT	AI	MA	RT	AI	MA
1	Alkaloid	-	-	-	+	-	-	+	-	-	+	-	-
2	Tannin	+	+	+	+	+	+	+	+	+	+	+	+
3	Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
4	Phlobatnin	-	-	-	-	-	-	-	-	-	-	-	-
5	Steroid	+	+	+	+	+	+	+	+	+	+	+	+
6	Terpenoid	+	+	+	+	+	+	+	+	+	+	+	+
7	Saponin	+	+	+	-	+	+	-	+	+	+	+	+
8	Reducing sugar	+	+	+	+	+	+	-	+	+	-	+	+
9	Glycoside	-	-	-	-	-	-	-	-	-	-	-	-
10	Anthraquinon	-	-	-	-	-	-	-	-	-	-	-	-

RT = *Ruellia tuberosa*, AI = *Aesculus indica*, MA = *Myrsine africana*.

Table 2. Total phenols, tannins, and flavonoid contents of methanol extracts of *R. tuberosa, A. indica,* and *M. africana*.

Plants	Total phenol content (mg $100g^{-1}$)	Total tannin content (mg $100g^{-1}$)	Total flavonoid content (mg $100g^{-1}$)
R. tuberosa	3.41±0.07b	0.74±0.06a	10.43±0.98b
A. indica	2.88±0.04c	0.53±0.05b	6.77±1.02c
M. africana	3.75±0.05a	0.31±0.03c	11.39±1.72a

Values in each column are the mean of three replication ± standard deviation. Mean in the column followed by different alphabets are significant at $p < 0.05$.

plants at different concentrations are described in Figure 1. The antioxidant potential of *R. tuberosa, A. indica*, and *M. africana* ranged from 10.78 to 77.41%, 11.78-80.41%, and 6.41 to 74.36%, respectively. It was observed that antioxidant potential increased with increasing concentrations of plant extracts. The %DPPH values of selected plants varied significantly at $p < 0.05$. The antioxidant potential of *R. tuberosa, A. indica,* and *M. africana* observed in this study is in close proximity to the previous studies [34-35]. A plethora of studies have revealed that the presence of free radicals in the body can lead to various chronic and degenerative diseases like diabetes, cancer, coronary heart disease, inflammation, stroke, etc. [36]. Antioxidants play a crucial role in preventing oxidative damage caused by free radicals. They inhibit the oxidation process by interacting with free radicals, catalytic metals, and chelating agents, as well as by acting as oxygen scavengers [37]. The DPPH is a rapid, consistent, and reproducible method extensively used for the search for antioxidants not only in plant extracts but also in pure compounds [38].

3.3. Antimicrobial Activity

The antimicrobial activities of different extracts of *R. tuberosa* (Figure 2**)** showed that ethyl acetate had the highest inhibitory activity against *B. atropheous* (69.36%) and *E. carotovora* (68.64%), followed by aqueous extract against *B. subtilis* (68.43%), ethanol extract against *B. atropheous* (68.35%), and hexane extract against *E. carotovora* (65.81%). Furthermore, ethyl acetate and aqueous extracts had no inhibitory potential against *K. pneumonia* and *E. carotovora*. The ANOVA showed that *R. tuberosa* extracts significantly ($p < 0.05$) inhibited the growth of selected microbial strains. The results obtained in this research work were in accordance with previous research that described the notable inhibitory potential of alcohol, chloroform, ethyl acetate, and water extracts against different opportunistic microbial strains [39]. In a similar study [40], promising inhibition of the methanol extract of the said plant was observed against *E. coli, P. aeruginosa, K. pneumonia,* and *B. subtilis,* which is supportive evidence of our findings.

The results of the percent inhibition potential of *A. indica* (Figure 3) revealed that ethyl acetate extract had a notable inhibitory zone against *S. aureus* (69.40%), *E. carotovora* (60.70%), and *P. aeruginosa* (50.89%), whereas ethanol, hexane, and aqueous extracts had >50% inhibitory activity

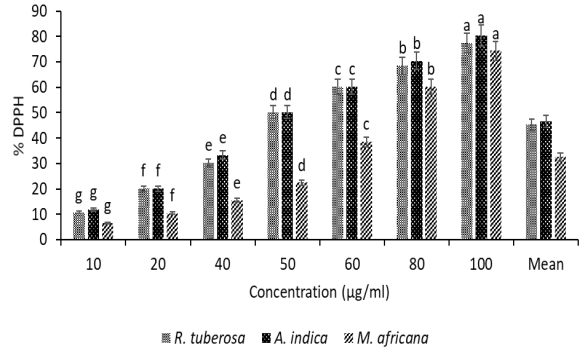

Fig. 1. Antioxidant activity of different concentrations of methanol extracts of *R. tuberosa, A. indica,* and *M. africana*.

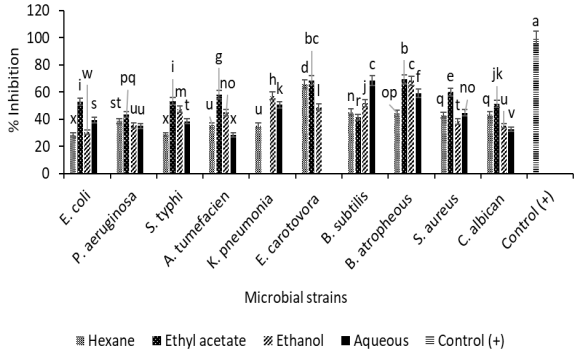

Fig. 2. Inhibitory potential of crude ethanol extract and fractions of *R. tuberosa* against pathogenic microbial strains.

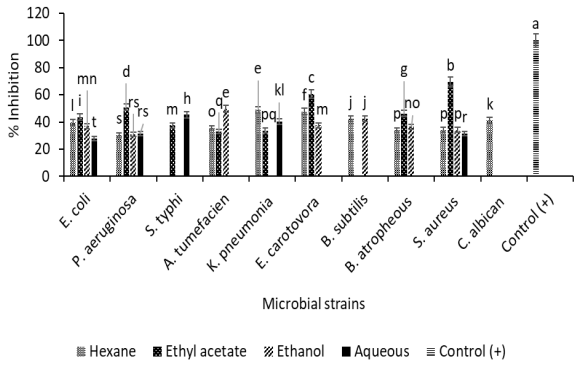

Fig. 3. Inhibitory potential of crude ethanol extract and fractions of *A. indica* against pathogenic microbial strains.

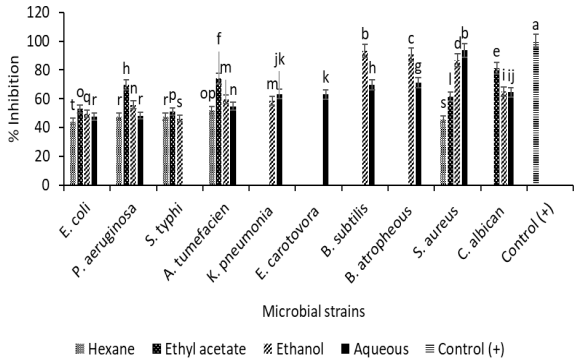

Fig. 4. Inhibitory potential of crude ethanol extract and fractions of *M. africana* against pathogenic microbial strains.

against the test microbial strains. It was observed that some microbial species showed resistance to the said plant extract and displayed no inhibition activity. The ANOVA showed that *A. indica* extracts significantly ($p < 0.05$) inhibited the growth of selected microbial strains. The results of our study are on par with earlier findings, which showed promising inhibitory zones of methanol and aqueous extracts of the said plant against *S. aureus* and *B. subtilis* [41]. Similarly, the highest inhibitory zones of 20.0 mm and 18.0 mm for ethanol and aqueous extracts of *A. hippocastenum* were noted against *S. mutans* and *S. sanguis* [42].

Likewise, the antimicrobial ability of *M. africana* (Figures 4) revealed that the aqueous extract had the highest inhibition potential against *S. aureus* (93.56%), followed by the ethanol extract, which showed a notable percent inhibition against *B. subtilis* (93.24%), and *B. atropheous* (90.49%). The said plant extracts showed good to moderate and low to no inhibitory activities against the test microbial strains. The ANOVA showed that *M. africana* extracts significantly ($p < 0.05$) inhibited the growth of selected microbial strains. Earlier studies showed that methanol, hexane, and chloroform extracts of *M. africana* had good to moderate and low to no activity against the test pathogenic bacterial strains [43]. In another research work, the hexane, chloroform, and ethanol extracts of the leaves, stems, and roots of *M. africana* were screened for an antimicrobial susceptibility test. The results showed that all extracts except hexane obtained from stem and root exhibited considerable inhibitory activity against the test microbial strains. In addition, chloroform extract has shown more antimicrobial activity than ethanol extract [44].

Moreover, the standard drugs, i.e., levofloxacin, ciprofloxacin, and clotrimazole, used as positive controls against gram-negative, gram-positive, and fungi, exhibited 100% inhibitory potential against the test microbial strains. Likewise, pure dimethyl sulfoxide used as a negative control did not inhibit the growth of selected pathogenic microbial strains (Figures 5).

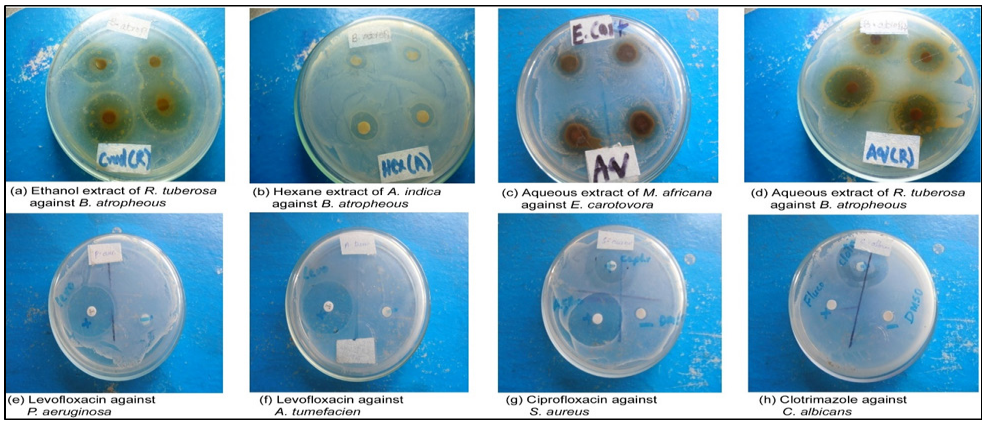

Fig. 5. Images of selected plant extracts and standard antibiotic against different microbial strains.

4. CONCLUSIONS

The results of the phytochemical analysis indicate that the selected plants are effective sources of various classes of secondary metabolites such as total phenols, total tannins, total flavonoids, etc. The selected plants exhibited notable antioxidant and antimicrobial activities, suggesting their potential as sources of antibiotics for treating various illnesses and for utilization in the pharmaceutical and cosmetic sectors. This study underscores the importance of isolating and identifying novel secondary metabolites to assess their effectiveness against different diseases.

5. CONFLICT OF INTEREST

The author has no conflict of interest.

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