Research Article

Bioactive Potential of Leaf Extracts (Tulsi, Curry and Ashoka) Through Total Phenolic Content, Antioxidant, Antimicrobial and Antifungal Analyses

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Abstract | The current research was carried out for the antimicrobial potential of leaves Tulsi, Curry and Ashoka, targeting two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) as well as two Gram-negative bacteria (*Vibrio parahaemolyticus* and *Escherichia coli*) and fungi viz., *Aspergillus niger, Alternaria alternate, Fusarium solani* and *Aspergillus flavus* were studied by Well diffusion method. Three solvent extracts Aqueous, Acetone and Methanol with concentrations (5,10,15 and 20%) was prepared. Our study showed that maximum zone of inhibition (ZOI) was observed against bacteria for *M. Koenigii* i.e., 22mm and 23 mm for fungi, while; *O. basilicum* and *S. asoca* plants have21 and 22mm MIZD. Methanol 20%has maximum antioxidant activity in *O. basilicum* whereas, in *S. asoca* and *M. koenigii* 20% aqueous showed maximum activity. Total phenolic content (TPC) 80%methanol exhibited high value in *O. basilicum* and *S. asoca* and aqueous 0% has a high TPC content in *M. koenigii*. The study of medicinal plants is an important area of research in modern medical science for better results and benefits to society and human safety. Use of medicinal plants for antimicrobial and secondary metabolites activities has gained tremendous attention from researchers.

Received | July 05, 2023; **Accepted** | September 05, 2023; **Published** | September 28, 2023
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Citation | Iqbal, H.M., S. Khurshid, S. Arif, Q.A. Akbar, S. Iqbal, S. Yousaf, K. Qureshi, A.K. Khan, A. Ahad, A.A. Siddique and N. Hamid. 2023. Bioactive potential of leaf extracts (Tulsi, Curry and Ashoka) through total phenolic content, antioxidant, antimicrobial and antifungal analyses. *Pakistan Journal of Agricultural Research,* 36(3): 277-284.
DOI | https://dx.doi.org/10.17582/journal.pjar/2023/36.3.277.284
Keywords | Antimicrobial, Antifungal, Antioxidant, Phenolic content, Well diffusion, Secondary metabolites

Introduction

Plants with medicinal properties serve as a significant reservoir of bioactive compounds including alkaloids, flavonoids, polyphenols, saponins, glycosides and tannins which have antioxidant, as nutraceuticals and food supplements, pharmacological properties have healing properties (Reyes *et al.*, 2020). Phytochemicals in medicinal plants contribute to various biological activities, encompassing not only

antifungal, antioxidant and antibacterial properties but also the initiation of cellular oxidation reactions. A diverse array of microorganisms coexist in a delicate equilibrium with the human body and its surrounding habitats (Al-Akeel *et al.*, 2018). However, when microbe proliferation becomes unregulated and swift, it can give rise to potentially hazardous issues (Scheepmaker et al., 2019).

Tulsi (*Ocimum basilicum)* or Holy Basil is an annual and perennial herbal plant belongs to the family Lamiaceae "Queen of plants". It is a shrubs native to the tropics of Asian and African countries and known as "mother medicine of nature" possessing medicinal value like anti-inflammatory property (Seyed *et al.*, 2021). Extracts demonstrated both antioxidant and antibacterial capabilities against both gram-positive and gram-negative bacteria. Extracts showed antioxidant and antibacterial activity against gram-positive and gram-negative bacteria (Eftekhar*et al.*, 2019). Curry leaves of *Morraya koenigii* grown in Asia belong to the family Rutaceae used in Asian cooking, due to their natural flavors. Leaves contain a wealth of vital phytochemicals, minerals and trace elements used to treat many diseases, including diabetics, cancers, etc. (Samanta *et al.*, 2018). *Saraca asoca* is commonly referred to as Ashoka, a member of the Caesalpiniaceous family (Fatema *et al.*, 2019) is used exhaustively as a herbal drug to cure several diseases to manage various disorders like cancer, skin infections bacterial infections (Devan and Warrier, 2021).

Current research planned to assess activity in scavenging free radicals, overall phenolic content and antimicrobial effects of Aq, Ace and MeOH leaf extracts of *O. basilicum, S. asoca* and *M. koenigii* against food-borne pathogenic bacteria and fungi, also to determine the minimum inhibitory concentration (MIC).

Material and Methods

Plant material

Leaves of *O. basilicum, S. asoca* and *M. koenigii* were collected from the garden area of Southern Zone Agriculture Research Center, University of Karachi, Pakistan. The leaves 200gm were washed with tap water air dried and ground into powder using an electric blender (Moulinex LM438 France) and placed in a closed jar for further analysis.

Extraction

For the leaves samples extraction, a method of Akwu *et al.* (2019) was slightly modified such as two hundred gram (200g) powdered leaves of *O. basilicum*, *S. asoca* and *M. koenigii* were macerated in methanol (MeOH), acetone (Ace) and water (Aq) for 24-48 hr. Subsequently, filtration was carried out using Whatman No 1 filter paper. The extracts were then subjected to air drying at room temperature and subsequently stored in a refrigerator at 5 °C for further analysis. Following the crude extract yield for each set of plant leaves was calculated using the formula provided below

Extract yield (%) = Mass of dried extract (g)/ Mass of leaf material (g) X 100

Antibacterial activity and antifungal activity

The antimicrobial and antifungal activity was investigated against four bacterial strains: *E. coli* (ATCC 8739), *S. aureus* (ATCC 43300), *B. subtilis* (ATCC 11778) and *V. parahaemolyticus* (ATCC 17802) and four fungal strains viz., *A. niger, A. flavus, A. alternate* and *F. solani*. A 9mm hole was punched with a disinfected cork borer. 70 µL from each extracts concentration were dispensed in each well. Plates were left for 18-24 hr at 35-37°C and ZOI was noted (Sadeq *et al.*, 2021).

Minimum inhibitory concentration (MIC) determination

The method of minimum inhibitory concentration was used to determine the lowest concentration that would show no visible growth. The plant extracts underwent a series of dilution steps according to the different concentrations (5, 10, 15 and 20%). Nutrient broth powder (bacterial) and potato dextrose powder (fungi) were used. In a sterile test tubes 10 mL of broth with 0.5mL of the extracts was added and a loopful of the suspension of organisms were added to the broth tube, shaken and incubated for 24 hr at 37°C for bacterial activity and 30°C for 48 hr for fungi after which the test tubes were observed for turbidity (Nyam *et al.*, 2020).

Mechanism of action exhibited by the extracts

The mode of action of the extract was visually analyzed. The test tubes with no growth showed full control of bacteria and the tubes with slight growth showed a little bit of control by the extract. Extract concentration with intense growth was considered to be no antimicrobial effect (Mohan *et al.*, 2016).

Antioxidant activity

Analysis of free radical scavenging activity was conducted using the DPPH method reported by (Coklar and Akbulut, 2017). A volume of 100 µL from the sample was combined with 2.7 mL of 0.1% methanol, followed by the addition of 200 µL of a 0.1% DPPH solution in methanol. The resulting mixture was incubated for 30 min in darkness. Control samples were prepared using identical amounts of methanol and DPPH solution. Absorbance was subsequently measured at 517 nm using a UV/Vis spectrophotometer. The percentage of inhibition was determined using the formula below:

$$\% \text{ inhibition} = \text{Absorbance (control)} - \text{Absorbance (sample)} / \text{Absorbance (control)} \times 100$$

Total phenolic content (TPC)

The determination of total phenolic content (TPC) followed the procedure outlined by Singleton *et al.* (1999) employing Folin-Ciocalteu's reagent. In a nutshell, 0.1 mL of the prepared extract was mixed with 0.5 mL of 10% Folin reagent and the mixture was allowed to stand for approximately 6 minutes. Subsequently, 1 mL of 7.5% Na_2CO_3 was added with thorough mixing. The resulting solutions were left to stand at room temperature for 2 h. After this incubation period, absorbance was measured at 765 nm using a UV/Vis spectrophotometer.

Results and Discussion

Extraction yield %

The extraction yields varied between 18%-41%. The minimal extraction yield emerged from Ace solvent extracts acquired from *O. basilicum*, while the maximum yields originated from the MeOH solvent extracts of *M. Koenigii* leaves. This variation in total yield could potentially arise from multiple factors, encompassing the plant material's, origin, collection site, drying methodology, moisture content and the possible interactions with various additional compounds often present alongside the extracted constituents, as suggested by (Kozłowska *et al.*, 2022). Table 1 displays the outcomes of the extraction yield obtained from the leaf materials utilized in this study.

Antibacterial activity

Aq extract showed slightly minimum inhibition as compared to MeOH and Ace extracts against microorganisms (Table 2). Results indicated that *M. koeniggi* gave18-21mm MIZD, *O. basilicum* showed 16-19mm and *S. asoca*15-18mm ZOI in MeOH and Ace, in Aq leaves extract produced 11-14mm mean inhibition of zone diameter against food borne bacteria which is comparable with a zone of inhibition exhibited by postive control antibiotic chloramphenicol 18-21 mm and negative control water 10-12 mm (Figure 1A). The antimicrobial efficacy is attributed to the capacity of medicinal plant extracts to induce lysis and degrade bacterial cell walls, ultimately disrupting bacterial activity. Among the leaf extracts, the highest level of activity was observed, possibly due to the presence of bioactive metabolites within the leaves (Jiang *et al.*, 2015). Notably, these extracts exhibited a stronger effect against gram-positive microorganisms compared to gram-negative bacteria, attributed to the absence of an outer lipid membrane protecting gram-positive bacteria in challenging conditions. These findings are consistent with previously reported outcomes (Mostafa *et al.*, 2018). *M. koenigii* extracts showcased greater activity among the three plant extracts, indicating that the size of the bacterial inhibition zone corresponded with extract concentration increments. It suggests that the extracts possess antibacterial substances that effectively hinder microbial growth. These results align with prior study reported by Devan and Warrier (2021).

Table 1: *Percentage yield of the crude leaves extracts of O. basilicum, S. asoca and M. koenigii.*

Solvents	Dried extract yield (g)			Percentage yield (%)		
	O. basilicum	***S. asoca***	***M. koenigii***	***O. basilicum***	***S. asoca***	***M. koenigii***
MeOH	3.2	2.9	4.1	32	29	41
Ace	1.8	2.1	2.2	18	21	22
Aq	2.5	2.7	2.8	25	27	28

Antifungal activity

Present study revealed that *M. koeniggi* gave 19-23mm inhibition zone while, *O. basilicum* 16-19mm and *S. asoca* 18-20 mm ZOI in MeOH, in Aq the leaves extract produced zone ranges from 9-13mm ZOI and in Ace *M. koeniggi* 18-20mm, *O. basilicum* produced 15-17mm while *S. asoca* gave 15-19mm MIZD targeting *A. niger, A. flavus, A. alternate* and *F. solani* which is comparable with a zone of inhibition exhibited by postivie control penicilin 20-23 mm and negative control water 09-11 mm (Figure 1B). The antifungal activities of *O. Basilicum* could be related to chemical compounds containing saponin, phenols

and alkaloids in the aqueous extract. Some studies state that leaf extract of *O. basilicum* completely inhibits fungal plant pathogens, such as *Botrytis, Fusarium* and *Rizoctonia* species (Mittal *et al*., 2018). *M. koenigii* exhibited efficacy against an extensive array of pathogenic fungi such as *Penicillium*, *Aspergillus* and *Fusarium* species. The bioactive compounds present in *M. koenigii* distinctly possess the capability to hinder mycelial growth, consequently enhancing its antifungal potential. Our results remain the best in comparison with this study (Tripathi *et al*., 2018). Seleshe and Kang (2019) observed MeOH showed maximum inhibition activity as compared to water extract against bacterial and fungal organisms.

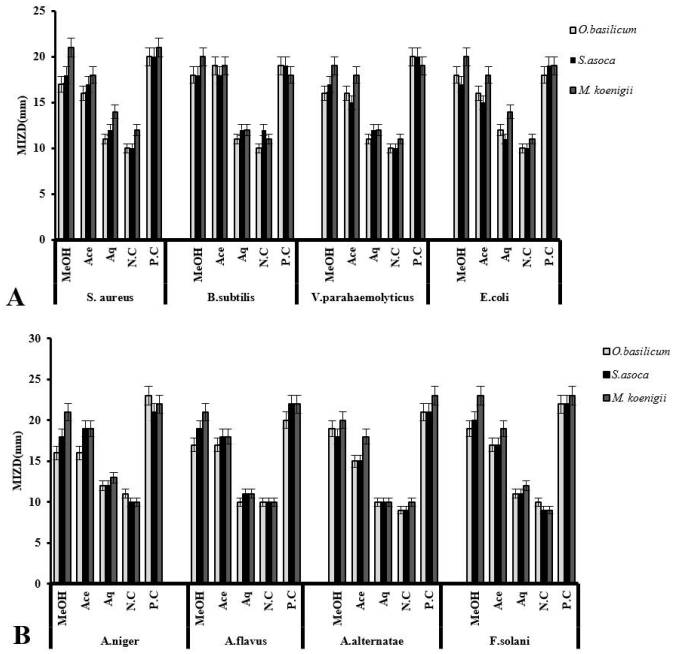


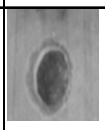
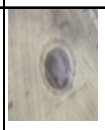
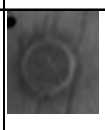
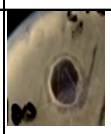
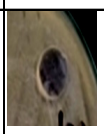
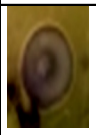
Figure 1: *Mean Inhibition Zone Diameter of various extracts against (A) bacterial strains; (B) fungal strains (N.C: Negative control; P.C: Positive control).*

Minimum inhibitory concentration (MIC)

The growth of the fungal species and food-borne bacterial species were significantly suppressed by the MeOH leaves extracts of *O. basilicum*, *M. Koenigii* and *S. asoca* (Table 3). At 0 and 5% concentrations test tubes were very cloudy in the MeOH extracts, except *S. asoca*. It showed cloudy representation against *B. subtilis* and *M. koenigii* showed cloudy against all 5 tested bacterial strains. *O. basilicum* remained cloudy in *B. subtilis* with 10% concentrations. *S. asoca* and *M. koenigii* showed slightly clear against *S. aureus*. All the test tubes were cleared at 15 and 20% concentrations in MeOH extracts shown (Table 3).

Curry leaves show MIC effects against *E. coil* and *S. aureus*. Based on the MIC test results against fungi

Table 2: *Plates displaying antibacterial and antifungal activity of MeOH crude extract from the leaves.*

Leaves extract	Concentration of extracts				
	5%	10%	15%	20%	
O. basilicum					Anibacterial Activity
S. asoca					
M. koenigii					
O. basilicum					Antifungal Activity
S. asoca					
M. koenigii					

represented in (Table 3) all the test tubes at 0% concentration were very cloudy in all of the three extracts and at 5% concentration leaves extracts showed effectiveness against all the tested fungi that shows cloudy to slightly clear results. All the test tubes were cleared at concentrations of 10, 15, and 20%. It was shown clearly that the higher the concentration of extracts of increases the mean diameter zone of inhibition. These MIC findings align with those previously reported by Sivananthan (2013). The varying levels of phytochemicals or secondary metabolites in different plant parts led to diverse reductions in microbial growth.

Radical scavenging activity

The Aq extracts contained high radical scavenging activity in comparison to MeOH and Ace extracts in *S. asoca* and *M. koenigii* while, in *O. basilicum* MeOH

has shown higher antioxidant activity. Obtained results was supported by Sablania *et al.* (2019) in which Aq extract of curry leaves showed higher activity than MeOH and Ace at 1000 µg/mL of extract concentration. In a study conducted by (Ali *et al.*, 2021), it was found that the MeOH extract exhibited the highest potency in terms of antioxidant radical scavenging activity at 93±0.65%, followed by ethanol at 81±0.13% (Truong *et al.*, 2019) reported Ace (66.1%); MeOH (50.7%) showed scavenging activity/total antioxidant activity curry leaves at 100µg/ml concentrations. Figure 2 illustrates DPPH radical scavenging activity of leaves extract of *S. asoca*, *O. basilicum*, and *M. koenigii*.

Table 3: *MIC of leaves extracts of O. basilicum, S. asoca and M. koenigii MeOH extract against the microbial cultures.*

Bacterial species	Extracts	Concentration mg/mL				
		0	50	100	150	200
E. coli	*O. basilicum*	+++	+++	+++	_	_
	S. asoca	+++	+++	+	_	_
	M. koenigii	+++	++	_	_	_
S. aureus	*O. basilicum*	+++	+++	+++	_	_
	S. asoca	+++	+++	++	_	_
	M. koenigii	+++	++	++	_	_
B. subtilis	*O. basilicum*	+++	+++	_	_	_
	S. asoca	+++	+++	+	_	_
	M. koenigii	+++	++	_	_	_
V. parahaemolyticus	*O. basilicum*	+++	+++	+++	_	_
	S. asoca	+++	+++	_	_	_
	M. koenigii	+++	++	_	_	_
A. niger	*O. basilicum*	+++	+	_	_	_
	S. asoca	+++	++	_	_	_
	M. koenigii	+++	+	_	_	_
A. flavus	*O. basilicum*	+++	++	_	_	_
	S. asoca	+++	+	_	_	_
	M. koenigii	+++	++	_	_	_
A. alternate	*O. basilicum*	+++	++	_	_	_
	S. asoca	+++	++	_	_	_
	M. koenigii	+++	++	_	_	_
F. solani	*O. basilicum*	+++	+	_	_	_
	S. asoca	+++	+	_	_	_
	M. koenigii	+++	+	_	_	_

+++, Very cloudy; ++, Cloudy; +, slightly; -, Clear

Pathak and Niraula (2019) reported that MeOH extract displayed a notable free radical scavenging activity of 71.42% at a concentration of 110µg/mL. The research conducted by (Mahirah *et al.*, 2018) similarly highlighted that MeOH extracts demonstrated the greatest DPPH scavenging activity at 92.6%, trailed by ethanolic extracts at 42.6%, and aqueous extracts. The antioxidant efficacy is contingent upon the quantity of phytochemicals present, including flavonoids, phenols, and others, which function to mitigate the generation of free radicals in oxidation reactions.

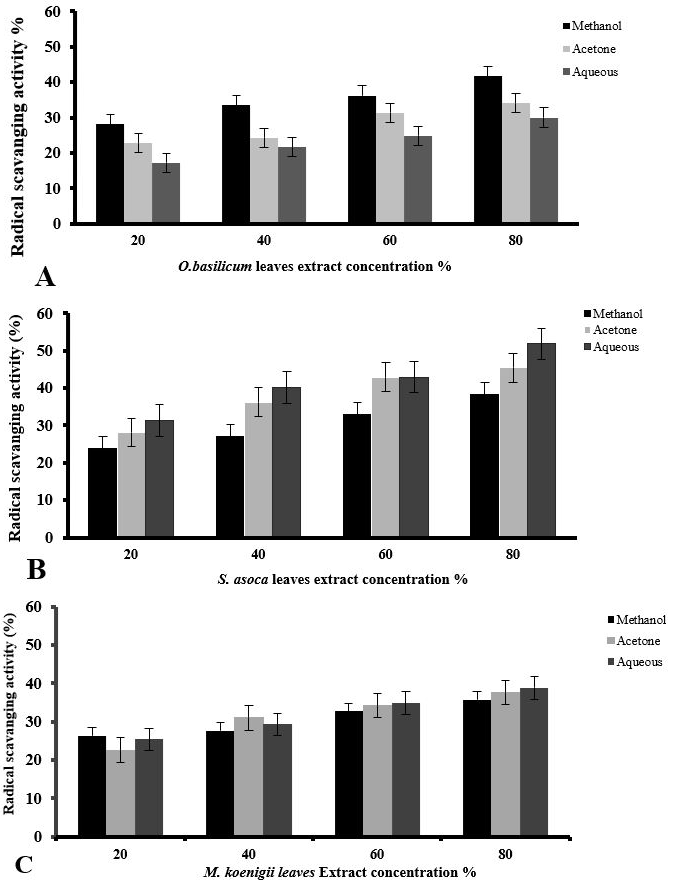


Figure 2: *DPPH scavenging activity of MeOH, Ace and Aq extracts of leaves of O. basilicum (A); S. asoca (B) and M. koenigii (C).*

Total phenolic content (TPC)

The results showed that extracting solvent had effects on TPC. Similar to the study of Bartariya *et al.* (2017) our results also showed that the MeOH extracts exhibited a high concentration of phenolics in *O. basilicum*, *S. asoca* in comparison to other solvents including water, hexane and acetone extracts while in *M. koenigii* Aq has a maximum value of TPC followed by extracts of MeOH and Ace (Table 4).

The current results for TPC showed that in *S. asoca* MeOH leaf extract contain high phenolic content than hexane. The quantitative assessment of phenolic content demonstrated that the methanol extract possessed a higher phenol concentration compared to the chloroform extract, our findings are in line with the research conducted by Bartariya *et al.* (2017).

Table 4: *Total Phenolic content in leaf extract of O. basillicum, S. asoca and M. koenigii.*

Conc. (%)	*O. basilicum*			*S. asoca*			*M. koenigii*		
	MeOH	Ace	Aq	MeOH	Ace	Aq	MeOH	Ace	Aq
5	25.7±0.13	15.3±0.16	10.7±0.15	24±0.13	23.9± 0.2	20.6±0.4	15.2±0.2	20.6±0.4	21.4±0.2
10	29.6± 0.08	20.6±0.4	15.4±0.1	28.8± 0.07	26.4±0.6	22.1±0.5	15.5±0.3	21.8±0.1	24.6±0.2
15	32.0± 0.19	23.4±0.2	17.7±0.1	31.6± 0.6	29.8±0.9	27.9±0.2	17.8±0.2	24.8±0.2	28±0.17
20	34.9± 0.06	28.0±0.14	21.8±0.1	37.9± 0.19	37.0±0.2	30.1±0.9	22.6±0.2	27.9±0.2	30.3±0.2

MeOH, Methanol; Ace, Acetone; Aq, Aqueous

The results were aligned with the findings of (Pathak and Niraula, 2019) which showed a high amount of TPC in *O.basilicum* where methanol > chloroform> hexane. Premi and Sharma (2017) suggested that aqueous extract had high extraction efficiency in *M. koenigii* leaves extracts which leads to higher total phenols as compared to acetone and methanol. These findings confirmed that solvents play an important role for the extraction of phenolic compounds.

The high amount of TPC in plant helps to remove free radicals and may directly contribute as an antioxidant compound. The phytochemical constituents such as phenols and several other secondary metabolites provide a defense system against many microorganisms and other insects.

Conclusions and Recommendations

Antimicrobial activity of *O. basilicum, S. asoca* and *M. koenigii* leaves have great importance for medicinal purposes. The outcomes of the study reveal that the O. *basilicum*, *S. asoca* and *M. koenigii* has active components such as phenol and possess antioxidant potential to control of bacteria: *E. coli*, *S. aureus*, *B. subtilis* and *V. parahaemolyticus* and fungi *A. niger, A. flavus, A. alternate* and *F. solani*. Our data reveal that leaf extracts of all three plants could be used as an effective alternative to chemicals based pesticides, fungicides and preservatives, are eco-friendly and produce less pollution in comparison to synthetic compounds. Also, leaves have great scavenging activity and are a good source of natural antioxidants. Outcomes from current research work signifying those leaves can be utilized for pharmaceutical nutraceutical and cosmeceutical applications.

Acknowledgment

The authors are thankful to their lab staff for assisting to carry out the research work.

Novelty Statement

This study marks the initial report of three medicinal plants; *O. basilicum*, *S. asoca* and *M. koenigii* leaves extracts, have active component phenol with high antioxidant potential that inhibit the growth of fungi and bacteria.

Author's Contribution

Hafiza Mehwish Iqbal: Designed, analyzed samples and wrote the manuscript (Equally).
Salman Khurshid: Statistical application, data analysis and wrote the manuscript (Equally).
Saqib Arif, Qurrat-Ul-Ain Akbar: Supervision andGave technical support and conceptualization.
Saba Iqbal: Provided assistance throughout the study and managed article.
Shahid Yousaf and Kainat: Provided assistance in reviewing the manuscript.
Aqeel Ahmed Siddique, Abdul Karim Khan, Abdul Ahad, Neelofar Hamid: Provision of samples and field activities, support in manuscript reviewing.

Conflicts of interest
The authors have declared no conflict of interest.

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