

Phytochemical Analysis of *Salvadora oleoides* and *Withania somnifera*: An Insight into Their Antioxidant and Antimicrobial Capabilities

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Abstract: *Salvadora oleoides* and *Withania somnifera* are the commonly found plants of Pakistan that are known to possess various medicinal properties. Present study was designed to evaluate the presence of various phytochemicals in these plants both qualitatively and quantitatively. This study was concluded with accessing the antioxidant and antimicrobial potentials of these phytochemicals against *E. coli*, *Shigella Spp*, *Aspergillus terreus* and *Aspergillus niger*. In addition qualitative analysis of phytochemical constituents i.e. alkaloids, glycosides, flavonoids, saponins, steroids, tannins and terpanoids and quantitative analysis of total proteins, sugars, reducing sugars, phenolics and flavonoids was performed. Plant extracts were also checked for the existence of antioxidant and antimicrobial activities. Results revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, flavonoids, saponins, steroids, tannins and terpenoids in both the plants. Quantification of few pharmaceutically important phytoconstituents showed the diversified response. Presence of antioxidants was confirmed in *S. oleoides* (2.14±SD mg/mL) in methanol extracts of leaves and water extract of leaves of *W. somnifera* (1.97±SD mg/mL). Methanolic extracts of leaves, bark and roots of both the medicinal plants showed inhibitory effects against both fungal and bacterial strains used. Our findings provide strong evidence that these medicinal plants possess phytoconstituents of pharmaceutical importance and thus may serve as an effective alternative to routine therapeutics.

Keywords: *Salvadora Oleoides*, *Withania Somnifera*, Phytochemicals, Antioxidant Activity, Antibacterial and Antifungal Activity, Pharmaceuticals

1. Introduction

Medicinal plants are potential source of providing certain phytochemicals known for antimicrobial and antioxidant properties. In developing countries, nearly 60 to 90% of the population use plant-derived medicines. Traditionally, herbal medicines are attributed to crude plant extracts used for the treatment of different human infectious diseases since old age [1-4]. Presently, their importance and usage in Unani, Ayurvedic medicine and pharmacological system is a well-established fact.

Salvadora oleoides Decne. (Salvadoraceae), also known as jall, Khabbar and Miswak is one of the most common tree of

Thar Desert of Pakistan. The oil-yielding potential of this tree makes it potential candidate for various pharmaceutical applications. Furthermore, this plant possesses good medicinal value and is used by locals for the treatment of various infections and / or diseases. Decoction of leaves proved its effectiveness to relieve cough, fever and a treatment of enlarged spleen. In addition, it is well-known to promote the expulsion of dead fetus from the uterus in cattle. Flowers of *S. oleoides*, in contrast are known to serve as blood purifier and nerve tonic. The leaf paste of this tree has proven to possess anti-inflammatory, analgesic, and anti-ulcer activity. Stem possesses an anthelmintic and diuretic activity. Root bark is used in the treatment of piles, whereas seeds are

effective to heal cough [5–11].

Withania somnifera (L.) Dunal (Solanaceae), commonly called as Aswagandha, winter cherry or Indian ginseng serves both as tonic and a sedative due to its adaptogenic properties [12, 13]. *W. somnifera* is known to possess anti-cancer properties against prostate, colon, lung, breast, leukemia, pancreatic, renal, head and neck cancer cells of humans [14–16]. Preclinical research and clinical trials support the use of *W. somnifera* for the treatment of various neurological conditions including anxiety, depression, cognitive disorders, senile dementia, neurodegenerative disorders (Alzheimer's and Parkinson's diseases) [17, 18, 21, 22], epilepsy and seizures [19, 20]. A number of studies have reported anti-inflammatory, anti-arthritis, spermatogenic and hepato-protective properties of this medicinal plant [23–27].

E. coli and *Shigella Spp* are known to be the major bacterial etiological agents responsible for diarrheal infections worldwide among humans [28, 29]. In contrast, *Aspergillus terreus* and *Aspergillus niger* are associated with an upper respiratory tract infection Aspergillosis which serves as a source of significant morbidity and mortality in humans [30]. Due to emerging threat of multidrug resistance strains treatment becomes critical and therefore, search of an alternative and safest therapeutics to cure such infections is pivotal. Studies on traditional medicinal plants and their antibacterial effect are frequently carried out in field of microbiology and pharmacology for development of a new antimicrobial agent to control growth of disease causing pathogens.

Present study was designed to focus on *Salvadora oleoides* and *Withania somnifera* plant species for the screening of the phytochemicals. Experimentations were further continued with the screening of antioxidant and antimicrobial potentials of these phytochemicals against *E. coli*, *Shigella Spp*, *Aspergillus terreus* and *Aspergillus niger*.

2. Materials and Methods

2.1. Collection and Identification of Plants

Mature plants of *Salvadora oleoides* and *Withania somnifera* were collected from garden of Institute of Biotechnology and Genetic Engineering, University of Sindh - Jamshoro. Collected plant specimens were authenticated by renowned taxonomist. Both plants were washed and cleaned with distilled water (D/W), followed by the separation of root, bark and leaves. The plant materials were then air dried separately at room temperature for three days until water molecules evaporated completely and all plant parts become well dried for grinding. The samples were then crushed into powder, using mechanical grinding machine, hence the effective contact of solvent with the sites on the plant materials can be enhanced.

2.2. Preparation of Plant Extracts

In order to screen primary and secondary metabolites from aforementioned samples, 5g of each powdered root, bark and leaves was separately dissolved in 100 mL of methanol. The sample flasks were kept at room temperature for 24 hours with

continuous stirring. Later, all samples were centrifuged at 6000 rpm for 15 minutes at 4°C. The supernatant was filtered through Whatman filter paper No. 1 and solvent was evaporated yielding crude extract. The same procedure was followed for water extraction of metabolites. Both water and methanol crude extracts (Final volume 50mL) were stored at -40°C for further phytochemical studies.

2.3. Qualitative Phytochemical Analysis

Both water and methanolic extracts of aforementioned plant parts were subjected to qualitative phytochemical screening of chemical secondary metabolites of alkaloids, glycosides, flavonoids, saponins, steroids, tannins and terpenoids [31–35].

2.4. Quantitative Phytochemical Analysis

Quantitative phytochemical analysis of methanolic and water extracts of leaves bark and roots of *S. oleoides* and *W. somnifera* included the screening of proteins, sugars, reducing sugars, phenolic and flavonoid compounds [36–40].

2.5. Determination of Antioxidant Activity

The antioxidant activity from methanol extract of *S. oleoides* (leaves bark and roots) and *W. somnifera* (leaves bark and roots) was determined by the method reported elsewhere [41]. For the reaction, 0.2 mL of test sample was combined with 2 mL of reagent solution (0.6M Sulphuric acid, 28mM Sodium phosphate, and 4mM Ammonium molybdate) in duplicate. Then tubes were incubated in the boiling water bath at 95°C for 90 minutes followed by the cooling at room temperature. Blank was prepared by adding D/W along with all reagents. Absorbance was determined at 695 nm using UV-visible spectrophotometer.

2.6. Determination of Antimicrobial Activity

Two bacterial strains (*E. coli* and *Shigella Spp*) and two fungal strains (*Aspergillus terreus* and *Aspergillus niger*) were used in this study. The bacterial and fungal isolates were first separately sub-cultured in a Nutrient broth (Oxoid) and Sabouraud's dextrose broth (Oxoid) respectively. All media tubes were incubated at 37°C for 18 hours. Both plant extracts were dissolved in 10% DMSO (Dimethyl sulfoxide) to a final concentration of 2 mg/mL. The antimicrobial activities were determined by Kirby-Bauer's disc diffusion method as per National Committee for Clinical Laboratory Standards recommendations [42]. Nutrient agar and Sabouraud's dextrose agar plates were separately cultured with inoculums of each bacterial and fungal isolate respectively using sterile cotton swabs. The discs of extracts placed and plates were incubated at 37°C for 24 hours. The average inhibition zone diameters were measured and recorded.

2.7. Statistical Analysis

Descriptive analysis, including mean, proportions, percentage as well as descriptive graphs and tables were used. Values are expressed as mean±SD.

3. Results and Discussion

3.1. Qualitative Phytochemical Analysis

This experiments was delineated to screen and analyze the presence of preliminary phytochemicals in *Salvadora oleoides* (methanolic and water extracts of leaves bark and roots separately) and *Withania somnifera* (methanolic and water extracts of leaves bark and roots separately). The results revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, flavonoids, saponins, steroids, tannins and terpenoids in both the plants (Tables 1 and 2).

These results can be explained by the fact that falvonoid compounds play a major role for medicinal plants to have a strong antioxidant powers. Family Salvadoraceae and Solanaceae plants *S. oleoides* and *W. somnifera* are well known to possess important phytochemicals that providing immunity to plants and other species and provide further properties to plant body. Hence, are responsible to increase the structural framework of these living organisms. Plant body produce phytochemicals as secondary metabolites works with dietary fibers and nutrients to defend against pathogenic effects.

Table 1. Qualitative screening of phytochemicals in different parts of *Salvadora oleoides*.

Phytochemicals	<i>Salvadora oleoides</i> (Leaves)		<i>Salvadora oleoides</i> (Roots)		<i>Salvadora oleoides</i> (Bark)	
	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract
Alkaloids	-	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	-
Saponins	+	+	-	-	-	+
Steroids	+	+	-	-	-	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+

Table 2. Qualitative screening of phytochemicals in different parts of *Withania somnifera*.

Phytochemicals	<i>Withania somnifera</i> (Leaves)		<i>Withania somnifera</i> (Roots)		<i>Withania somnifera</i> (Bark)	
	Methanol Extract	Water Extract	Methanol Extract	Water Extract	Methanol Extract	Water Extract
Alkaloids	-	+	+	+	+	+
Glycosides	-	+	-	+	-	+
Flavonoids	-	+	+	-	+	-
Saponins	+	-	-	+	+	-
Steroids	-	-	-	+	-	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	-	+

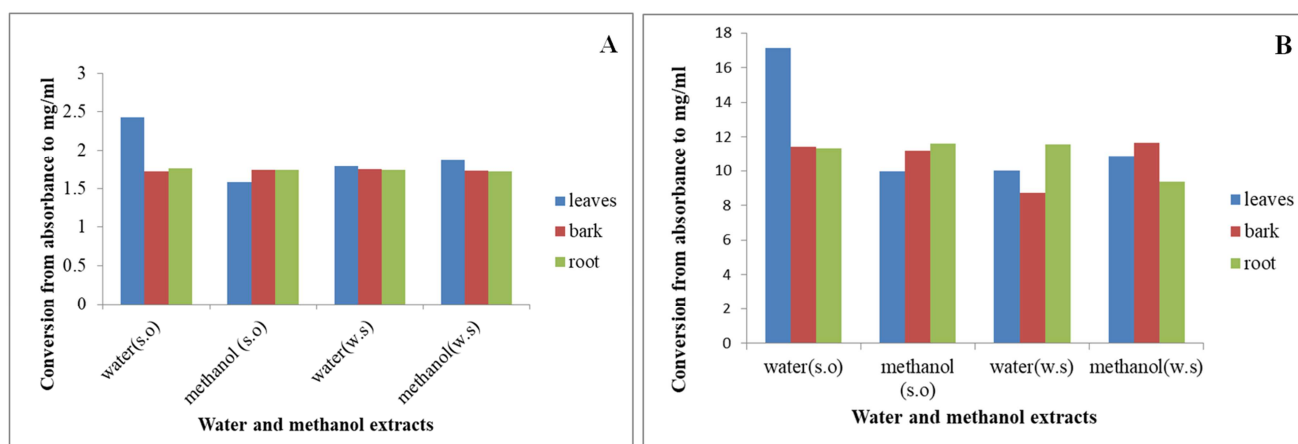


Figure 1. Quantitative phytoconstituents analysis of *Salvadora oleoides* and *Withania somnifera*. (A) Quantification of total proteins; (B) Quantification of total sugars.

3.2. Quantitative Phytochemical Analysis

Another set of experiments was conducted to quantify the different phytochemicals in aforementioned plants.

Interestingly, results indicated that in *S. oleoides* maximum quantity ($2.43 \pm \text{SD}$ mg/mL) of total proteins was obtained in water extracts of leaves, whereas methanolic leaf extract yielded the minimum quantity ($1.58 \pm \text{SD}$ mg/mL). In *W.*

somnifera maximum total proteins ($1.87 \pm \text{SD}$ mg/mL) were found in methanolic leaf extract whereas minimum quantity ($1.72 \pm \text{SD}$ mg/mL) was quantified in methanolic root extract (Figure 1A). Furthermore, water extracts of leaves of *S. oleoides* revealed to possess the maximum quantity of total sugar ($17.12 \pm \text{SD}$ mg/mL), whereas the minimum quantity ($9.95 \pm \text{SD}$ mg/mL) obtained in methanolic leaf extract. In contrast, methanolic bark extract of *W. somnifera* showed maximum total sugars ($11.65 \pm \text{SD}$ mg/mL) whereas minimum ($8.73 \pm \text{SD}$ mg/mL) was quantified into water bark extract of same plant (Figure 1B).

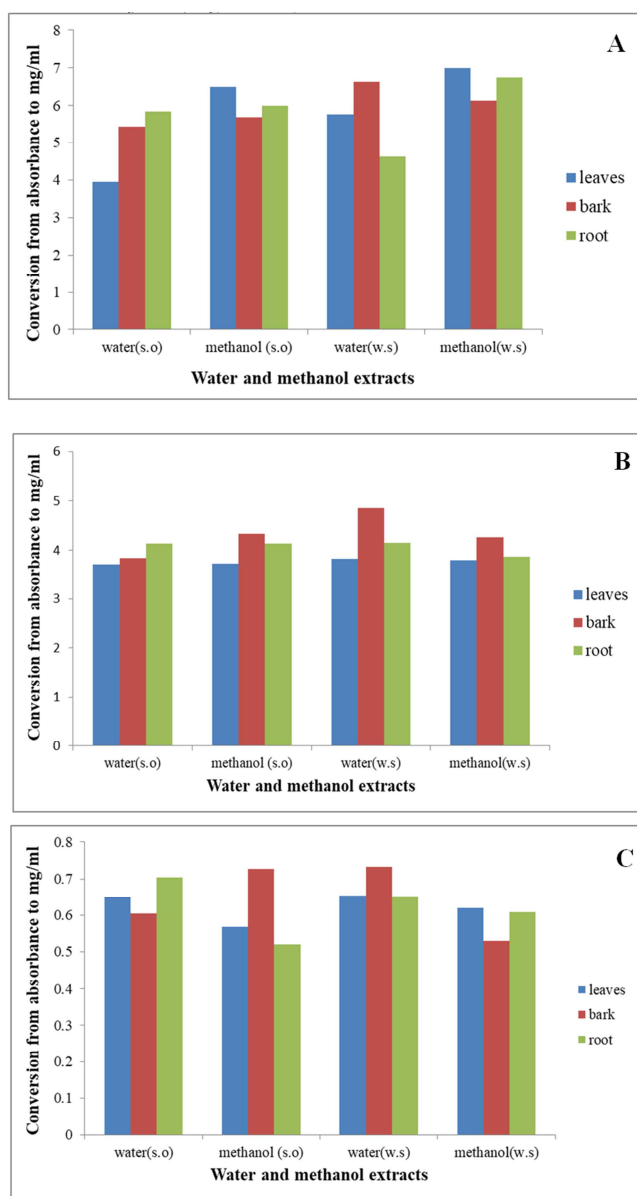


Figure 2. Quantitative phytoconstituents analysis of *Salvadora oleoides* and *Withania somnifera*. (A) Quantification of total reducing sugars; (B) Quantification of total phenolic compounds; (C) Quantification of total flavonoids compounds.

The results of quantification of total reducing sugars highlighted the presence of maximum quantity in methanolic extract of leaves ($6.49 \pm \text{SD}$ mg/mL) of *S. oleoides*. Whereas

water leaves extract of *S. oleoides* yielded the minimum quantity of reducing sugars ($3.95 \pm \text{SD}$ mg/mL). Conversely, methanolic leaf extract of *W. somnifera* yielded maximum quantity of ($7.00 \pm \text{SD}$ mg/mL) while minimum amount ($4.62 \pm \text{SD}$ mg/mL) was quantified into water root extract (Figure 2A).

Moreover, analysis of results of the quantification of phenolic compounds in *S. oleoides* and *W. somnifera* revealed diversified response. It was significant to note that maximum quantity of phenolic compounds ($4.33 \pm \text{SD}$ mg/mL) was obtained in methanol extracts of bark of *S. oleoides*, whereas the minimum quantity ($3.71 \pm \text{SD}$ mg/mL) was evaluated in water extract of leaves. In addition, water extract of bark of *W. somnifera* showed the presence of maximum quantity ($4.85 \pm \text{SD}$ mg/mL) of phenolic compounds. While, minimum quantity ($3.79 \pm \text{SD}$ mg/mL) was quantified in methanol leaves extract of same plant (Figure 2B). In another set of experiments flavonoid compounds were quantified in *S. oleoides* and *W. somnifera*. It is to worth mention herein that maximum quantity of phenolic compounds ($0.727 \pm \text{SD}$ mg/mL) were obtained in methanol extracts of bark of *S. oleoides*. Conversely, methanol root extract highlighted the presence of minimum quantity of phenolic compounds ($0.52 \pm \text{SD}$ mg/mL). Comparative analysis revealed that *W. somnifera* possess maximum amount ($0.733 \pm \text{SD}$ mg/mL) in water extract of bark whereas minimum quantity ($0.529 \pm \text{SD}$ mg/mL) was quantified into methanol bark extract (Figure 2C).

Keeping in view all aforementioned results can be explained by the fact that *S. oleoides* contain alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids and other phytochemicals like carbohydrates, proteins, phenolic and flavonoid compounds. These phytochemicals have proved to be the reason of various therapeutic values of this plant [43]. Moreover, roots of *Salvadora* plants locally known as Miswak, have been used for centuries as oral hygiene tool in many parts of the world. Fruits are sweet and usually enjoyed by local people. Conversely, *W. somnifera* also contain high amount of phytochemicals that helps against different diseases. *W. somnifera* possesses antioxidant, anxiolytic, adaptogen, memory enhancing, anti-parkinsonian, anti-venom, anti-inflammatory, anti-tumor properties. Various other effects like immune-modulation, hypo-lipidemic, antibacterial, cardiovascular protection, sexual behavior, tolerance and dependence have also been well studied.

The results of the present study can be further explained by the fact that the phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [34]. Nonetheless, the results of the present study are very encouraging and significantly indicate that both of these herbs i.e. *S. oleoides* and *W. somnifera* should be studied more extensively to reveal other potential therapeutic effects.

3.3. Presence of Antioxidant Activity Potentials

This experiment was delineated to evaluate the antioxidant activity in *S. oleoides* and *W. somnifera*, methanolic and water extracts of leaves bark and roots separately. Results of this experiment significantly highlighted the presence of antioxidant potentials in *S. oleoides* via yielding maximum quantity ($2.14 \pm \text{SD}$ mg/mL) in methanol extracts of leaves. Whereas the minimum quantity ($1.51 \pm \text{SD}$ mg/mL) obtained in methanol bark extract. Conversely, *W. somnifera* yielded maximum quantity ($1.97 \pm \text{SD}$ mg/mL) of antioxidants in water extract of leaves. While, minimum quantity ($1.45 \pm \text{SD}$ mg/mL) was quantified into water bark extract (Figure 3).

The possible explanation of these significant results is the presence of profound quantities of flavonoids and phenolic compounds (Tables 1 and 2, Figure 2B and 2C) in the plants under consideration. Extensive literature review revealed that several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [34, 44, 45]. This can be further explained by the fact that natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc [46]. These results can be further explained by the fact that polyphenol are the major plant compounds which are commonly found in both edible and medicinal plants and are reported to have multiple biological effects, including antioxidant activity. Their antioxidant activity is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers, which plays an important role in adsorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides. The antioxidant constituents of plant materials are known to be

important in maintenance of human health and protection from coronary heart disease and cancer.

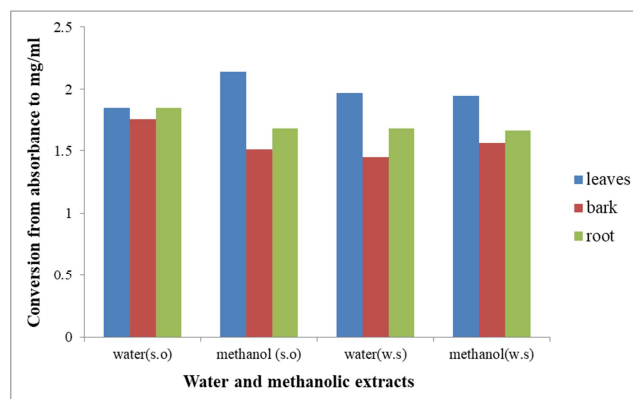


Figure 3. Antioxidant activity of *Salvadora oleoides* and *Withania somnifera* (mg/mL).

3.4. Presence of Antimicrobial Activity Potentials

Another set of experiments was conducted to evaluate the antimicrobial activity in *S. oleoides* and *W. somnifera*, methanolic and water extracts of leaves, bark and roots separately. It was interesting to note that methanolic extracts of leaves, bark and roots of both the medicinal plants showed inhibitory effects against both fungal and bacterial strains used. Conversely, water extract of leaves and roots of *W. somnifera* showed moderate inhibition of bacterial and fungal strains however, root extract was found unable to kill any of the microorganism. Similarly, water extracts of leaves, bark and roots of *S. oleoides* showed no effect on microorganisms used (Table 3).

Table 3. Antibacterial and antifungal activity profile of *salvadora oleoides* and *Withania somnifera*.

Parts of plants examined	Bacterial and fungal strains used	<i>Salvadora oleoides</i>		<i>Withania somnifera</i>	
		Methanol Extract	Water Extract	Methanol Extract	Water Extract
Leaves	<i>Aspergillus terreus</i>	20	0	20	08
	<i>Aspergillus niger</i>	15	0	12	05
	<i>E. coli</i>	16	0	10	02
	<i>Shigella Spp</i>	15	0	13	0
	<i>Aspergillus terreus</i>	12	0	05	0
Roots	<i>Aspergillus niger</i>	11	0	12	0
	<i>E. coli</i>	09	0	10	0
	<i>Shigella Spp</i>	06	0	07	0
	<i>Aspergillus terreus</i>	14	0	12	02
	<i>Aspergillus niger</i>	08	0	12	05
Bark	<i>E. coli</i>	05	0	10	05
	<i>Shigella Spp</i>	03	0	0	0

Numbers represent average inhibition zone diameters (mm)

The results of the present study can be explained by the fact that medicinal plant mediated antimicrobial activity can be attributed not only to a single bioactive principle but also in concert action with other compounds. Moreover, the chemical structures of the antimicrobial agents found in higher medicinal plants belong to secondary metabolites such as flavonoids, and phenolic acids. Therefore, medicinal plant extracts being great sources of phenolic compounds represent the highest antimicrobial activities may be considered as a good alternative of the routine use of

antibiotics. Thus these plants may serve as good addendum in the standard therapeutics in the course of infections caused by *E. coli*, *Shigella Spp*, *Aspergillus terreus* and *Aspergillus niger*.

4. Conclusion

Taken altogether, present study significantly proves that *Salvadora oleoides* and *Withania somnifera* plants found in Pakistan possess a wide variety of phytochemicals having

strong antioxidant and antimicrobial properties. Nevertheless, these natural antimicrobials and antioxidants could have a great importance to be used as a therapeutic agent in the prevention of devastating diarrheal and fungal infections. Furthermore, results of the present study serves as an addition to the existing medicinal properties of these plants. In addition, phytochemicals of these plants may further be screened for other lethal infections. Moreover, genetic variation studies may be carried out on same plants to enhance the production of biochemical, phytochemical and pharmacological compounds of pharmaceutical significance that helps against different diseases.

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