Available at www.ijcasonline.com ISSN:2349 – 0594

International Journal of Modern Chemistry and Applied Science

International Journal of Modern Chemistry and Applied Science, 2016, 3(3),421-426

Antimicrobial, Antioxidant and Anthelmintic Activities of the Essential Oils and methanol Extract of *Artemisia Absinthium*

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Abstract: *Artemisia absinthium* is a medicinal plant used by traditional herbal practitioners in whole part of the world for treating several diseases. This study was carried out to examine the biological activity of essential oil and methanol extract of *Artemisia absinthium*. The oils of plant were evaluated for their antimicrobial activity against both Gram-positive bacteria and Gram-negative bacteria by using a disc-diffusion method and for their antioxidant activity by measuring the hydrogen peroxide scavenging assay and phosphomolybdenum assay. Essential oil was also tested for anthelmintic activity against *Lumbricus terrestris*. The antimicrobial test results showed that the oil had a potential antimicrobial activity against all tested microorganisms with minimum inhibitory concentration (MIC) ranging between 0.097 and 0.39 mg/mL. Essential oil showed maximum zone of inhibition and minimum zone of inhibition against *Staphylococcus aureus* (ATCC-29523) and *Salmonella typhimurium* (ATCC-14028). The finding showed that oils of plant has good scavenging activity against both H_2O_2 and phosphomolybdenum methods. Oils of the plant also showed good anthelmintic activity in dose dependent manner. The results of the study suggested that the *Artemisia absinthium* oils have great potential as natural medicine for microbial, parasitic infections and cancers.

Keywords: *Artemisia absinthium L*., anti-microbial activity, antioxidant and anthelmintic activity.

Introduction

In recent years, microbial contamination of foods and drinking water causes different types of diseases. The consumption of microbes-infected foods is a serious challenge and threat for the health of the consumers[1]. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. The emergence of resistant Gram-negative bacteria presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity. Bacterial resistance to antimicrobial agents is a medical problem with public health, socioeconomic, and even political implications. Strategies to improve the current situation include research in finding new and innovative antibacterial antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms. In developing countries, due to the cost of efficient, antibacterial a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases[2]. Therefore, the increase food borne diseases has required the urgency to find new natural sources nontoxic antibacterial compounds.

Helminthiasis a parasitic infection still considered as the major cause of ill health of number of peoples throughout the world especially peoples from deprived communities of undeveloped countries with poorer sanitary and health facilities, because it is mostly caused and spread through to environmental contamination and transmission. This parasitic infection is also responsible for increasing the mortality and morbidity day by day all over the world. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances.

Humans are constantly exposed to free radicals created by electromagnetic radiation from the man made environment such as pollutants and cigarette smoke. These free radicals are responsible for causing a wide number of health problems, which include cancer, aging, heart diseases, and gastric problems etc[3]. In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants prevent oxidative damage by free radical may prevent the occurrence of disease, cancer, disorder and aging. The study of such an activity or any research leading to that discovery would pave the first steps towards solving the problems stated above.

Thus, the aims of this study were to investigate the antioxidant, anthelmintic and antimicrobial activity of essential oil from aerial part of *A. absinthium.*

Materials and Methods

Plant material collection

Aerial parts of *Artemisia absinthium Linn* was collected during spring (March) 2014 from different part of Mekoni, Tigray regional states of the northern part of Ethiopia. The botanical identification of plant sample was carried out by Addis Ababa University national herbarium, Addis Ababa, Ethiopia and voucher specimens (Herbarium No. CNCS-CN-No-001) were deposited at the institute.

Extraction of essential oil

First the collected plant parts was washed by tap water in order to remove dust and other contamination then air dried at room temperature in a dust free environment. They were pulverized into fine powder by using pestle and mortal. The air-dried sample (150 g) was subjected to hydro distillation using a Clevenger-type apparatus for 4 hour to obtain the essential oils. The oil was removed every one hour from the distillation apparatus to decrease evaporation of the oil due to heat. After the distillation was over, the oil was collected, filtered, weighed and kept in a stopper bottle. The oil extracted from each sample was found containing

fractions of water, which was removed by adding small amount of anhydrous sodium sulfate and kept in refrigerator at 4 ^{o}C for further analysis.

Extraction crude oils

The aerial parts of the plant were cleaned, shade dried and pulverized to powder using mortar and pestle. Required quantity of powder (100 g) was weighed and transferred to three different Stoppard flasks, and treated with methanol until the powder is fully immersed. The flasks were shaken every hour for the first 6 hour and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extracts were filtered. The extracts were collected and evaporated to dryness by using a vacuum distillation unit. The final residues thus obtained were then subjected to biological activity.

Determination of antioxidant activity

Phosphomolybdenum assay

The antioxidant activity of essential oil and methanol extract were evaluated by phosphomolybdenum method according to the procedure described in[4].

a. Hydrogen peroxide-scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method described in[5].

Determination of antimicrobial activity

Test organism

The antibacterial activity of the methanoic extract and essential oil was determined against two Gram positive and two Gram-negative microorganisms. The Gram-positive organism includes *Streptococcus bovies* (ATCC-33317) and *Staphylococcus aureus* (ATCC-29523) and Gram-negative organism include *Salmonella typhimurium* (ATCC-14028) and *Escherichia coli* (ATCC-35218). All microorganism used in the agar disc diffusion method were obtained from Institute of veterinary medicine of Mekelle University.

Preparation of media

Culturing media used for antibacterial assay was Mueller Hinton agar for growth respective bacteria. Mueller Hinton agar media was prepared in a conical flask by dissolving 38 g of powdered agar media in 1 liter of distilled water. Flask was heated on open flame to dissolve the medium completely and then sterilized the medium in an autoclave at 121°C temperature for 15 min.

Determination of anthelmintic activity

Test parasites (worms) used for the study were *Lumbricus terrestris* belongs to family of *Lumbricidae*. Parasites used under study were collected form moist soil from Mekelle university main campus and authenticated by biology department, Mekelle University. The worms used for the study were uniform in size. The worms used for the study were uniform in size. Numbers of past studies involving evaluation of anthelmintic activity recommends the use of *Lumbricus terrestris* model test parasites for screening anthelmintics and it has been reported that substances which have toxic effect against earthworms also have toxic effects against most of the worms by causing primary irritation[6] which leads to paralysis to the worms that results in the withdrawal of the worm outside the body.

Evaluation of anthelmintic activity using *Lumbricus terrestris*

Anthelmintic activity was evaluated by using the common method of evaluation of anthelmintic activity as used in previous studies[6, 7, 8] based on the evaluation of anthelmintic activity of plant extract. Albendazole solution was used as reference standard drug and dimethyl sulfoxide (DMSO) as control. After pouring the essential oil and methanol extract worms were placed in plates and plates were keep under observation until the death of worms. Observations were recorded in the form of time required for consecutive attacks of paralysis till the death of earthworm and at the end time required for complete death of worm was noted; death of worm has been confirmed by pointing with needle.

Result and Discussion

Antioxidant activity of the oils of *Artemisia absinthium*

Total antioxidant capacity

The present study shows that methanoic extract and essential oil of *Artemisia absinthium* exhibited increased antioxidant activity or decreased prooxidant activity with increasing concentration.

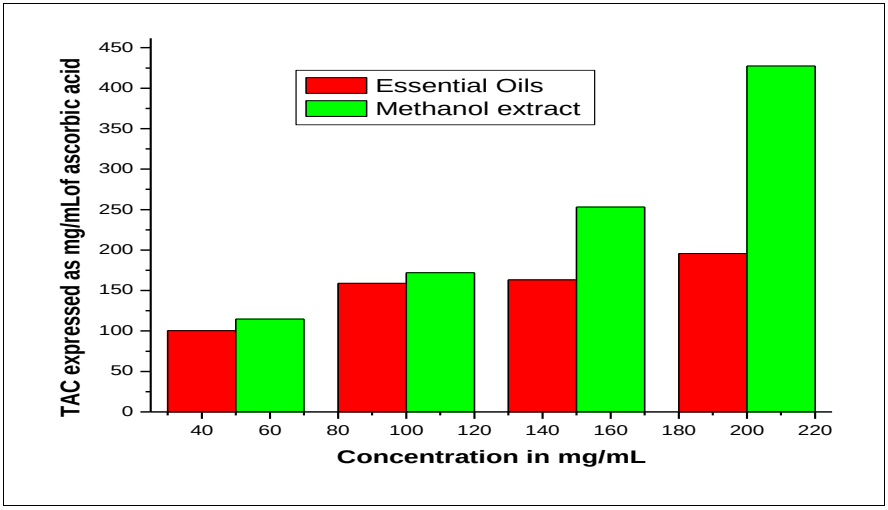

Figure-1 Total antioxidant capacity of *A.absinthium* expressed in ascorbic acid equivalents (mg ascorbic acid/g).

Based on the above graph, addition of the various extracts of *A.absinthium* showed that methanoic extract was more effective in reduction of Mo (VI) to Mo (V) while the lowest effects were shown by essential oil. The possible explanation for methanol extract having high total antioxidant capacity is due to high flavonoids content as flavonoids plays an important role as antioxidants in living systems due to the presence of hydroxyl groups within their

structure. The possible reason for essential oil having low total antioxidant capacity is due to the presence of small number of hydroxyl bearing compounds. This is an evidenced for presence of small phenol content in GC-MS analysis of the essential oil of *Artemisia absinthium*. Results of total antioxidant analysis are in agreement with the earlier studies where the methanol extract of aerial part of *Artemisia absinthium* was reported to possess reductive ability[9]. This is the opposite of earlier studies, in which the water extract of *A. absinthium* high antioxidant capacity than methanoic extract[10]. In spite of methanoic extract have highest antioxidant activity, both essential oil and methanoic extract of *A.absinthium* has capacity to reduce Mo (VI) to Mo (V) when used in a higher amount. It was evident, that *A.absinthium* did show reductive potential and could serve as electron donors, terminating the radical chain reaction.

According to these results, methanoic extract was confirmed as the most important contributor to the overall antioxidant effectiveness of plant, as previously suggested by the most of antioxidant assays performed in this study. Further it can be generalized on the basis of previous studies on *A.absinthium* and present results that higher altitude medicinal aromatic plant act as an importance antioxidant.

H_2O_2 scavenging activity

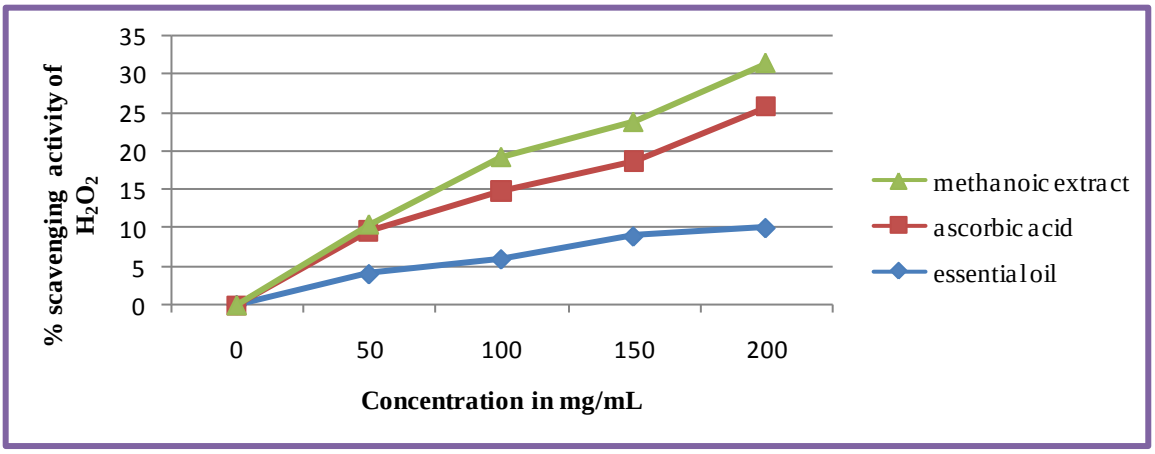

Figure-2 Hydrogen peroxide (H_2O_2) scavenging activity of *A.absinthium* and vitamin- C

In the present study, the test samples exhibited effective hydroxyl radical scavenging capacity at all concentration points and this may be attributed to the presence of phenols and tannins in *Artemisia absinthium*, which could donate electrons, thereby neutralizing it into water. The hydrogen peroxide scavenging capacity of the test samples increased with increase in the concentration (Figure-2). This indicates that *A. absinthium* products were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The hydrogen peroxide scavenging power of the test samples followed the order of methanol extract > ascorbic acid > essential oil. This showed that methanoic extract of *Artemisia absinthium* had highest hydroxyl radical scavenging effect and was most potent than ascorbic acid standard. The remaining water extract (essential oil) of wormwood possesses the lowest hydroxyl radical scavenging effect, which indicated that the important antioxidative compounds are present in very small amounts. Results of hydrogen peroxide scavenging analysis are in agreement with the earlier studies where the methanol extract of aerial part of *Artemisia absinthium* was reported to possess high hydroxyl radical scavenging effect[9].

Antimicrobial activities of the oils of *Artemisia absinthium*

As shown in these tables, the essential oil and methanol extract of *A. absinthium* exhibited considerable antibacterial activities against the majority of food borne pathogens. The essential oil of *A. absinthium* inhibited the growth of all microorganisms tested, while *A.absinthium* produced inhibition zones lower than or that of the standard antibiotic amoxicillin against all tested microorganisms.

Antibacterial activity showed that, the inhibition zones were found increased considerably when the concentration of the essential oil rate increased. Therefore, it can be said that quantity of the oil was important for inhibition effect. Among all Gram-positive bacteria growths, the maximum zone of inhibition was recorded against *staphylococcus aureus* i.e. 15.1 mm, followed *streptococcus bovies* i.e.14.67 mm. On other hand, two different Gram-negative bacterial strains were tested and among these microorganisms, *Escherichia coli* showed maximum zone of inhibition i.e. 14.5 mm, followed by *Salmonella typhimurium* i.e.14.25 mm. The minimum zone of inhibition was recorded against the *Salmonella typhimurium* strain i.e.14.25 mm. The highest value of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) was 0.39 and 1.56 mg/mL recorded in gram-negative strain *Escherichia coli* followed by Gram-negative strain *Salmonella typhimurium*, showed 0.39 and 0.78 mg/mL respectively (Table-1). Thus, different inhibitory effects of *A. absinthium* may be attributed to the differences in the biological properties of the main compounds in the oil. The wide antibacterial spectra of *A. absinthium* oils may also be attributed to their relatively high content of oxygenated sesquiterpene.

From these it can generalized that the essential oil showed maximum zone of inhibition, minimum inhibition concentration and minimum bactericidal concentration against *Staphylococcus aureus* and *Escherichia coli* respectively, which indicate that *A.absinthium L.* essential oil has capacity to inhibit the growth of both Gram-negative and Gram-positive bacterial strains when used in a higher amount. Therefore, based on the present results *A.absinthium* is higher altitude medicinal and aromatic plants acts as an important anti-microbial agent against many Gram- negative and Gram-positive bacterial strains.

The methanol extract of *A. absinthium* inhibited the growth of all microorganisms tested, while *A.absinthium* produced inhibition zones lower than or that of the standard antibiotic amoxicillin against all tested microorganisms.

Antibacterial activity showed that, the inhibition zones were found increased considerably when the concentration of the methanol extract rate increased. The tested extracts of *A.absinthium* aerial part possessed antibacterial activity against both Gram positive and Gram-negative bacteria. *Streptococcus bovies* strains showed more sensibility to those extracts compared with *Salmonella typhimurium, Escherichia coli* and *Staphylococcus aureus* strains.Results showed that antibacterial activities of the methanol extract have not remarkable different between Gram positive and Gram-negative bacteria. All the essential and methanol extract oils tested were subjected to MIC and MBC studies against all the microorganisms. The results in Table-3 interpreted as the lowest concentrations that inhibit the visible microbial growth and lowest concentrations that stop the growth of single bacterial colony. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values for bacterial strains, which were sensitive to essential oils of *Artemisia absinthium*, were in the range of 0.097-0.39 mg/mL and 0.19-1.56 mg/mL (Table-3). Based on these result it is possible to conclude that the essential oil have a stronger MIC and MBC as compared to the methanol extract tested.

Table-1 Anti-microbial activity of essential oil of *A.absinthium L.* against Gram-positive and Gram-negative bacteria strains

No.	**Nature of Bacterial strains**	**Microorganisms (Bacterial source Number)**	**Diameter of inhibition zone (mm) of essential oil Concentration used for anti-microbial analysis (mg/mL) (n = 3)**				**Control +ve (n = 3)**
							Amoxicillin
			25	**50**	**75**	**100**	**30 µg**
1	Gram +ve	*Staphylococcus aureus* (ATCC-29523)	10.25 ± 0.96	13.67 ± 0.57	14 ± 0.82	15.1 ± 0.90	18.67 ± 0.17
2		*Streptococcus bovies* (ATCC-33317)	9.5 ± 0.58	11.5 ± 0.13	13.33 ± 0.15	14.67 ± 0.58	18.5 ± 0.47
3	Gram - ve	*Salmonella typhimurium* (ATCC-14028)	8.87 ± 0.83	11 ± 1.0	13.33 ± 0.57	14.25 ± 0.96	16.9 ± 0.65
4		*Escherichia coli* (ATCC-35218)	10 ± 0.82	10.75 ± 0.96	11 ± 0.13	14.5 ± 0.71	17.33 ± 0.12

Values are means of triplicate determination ± standard deviation of mean

Table-2 Anti-microbial activity of methanolic extract of *A.absinthium L.* against Gram- positive and Gram-negative bacteria strains

No.	**Nature of Bacterial strains**	**Microorganisms (Bacterial source Number)**	**Diameter of inhibition zone (mm) of crude oil Concentration used for anti-microbial analysis (mg/mL) (n = 3)**				**Control +ve (n = 3)**
							Amoxicillin
			25	**50**	**75**	**100**	**30 µg**
1	Gram +ve	*Staphylococcus aureus*(ATCC-29523)	8.25 ± 0.43	9.33 ± 0.58	11.33 ± 0.57	12.5 ± 0.13	18.87 ± 0.55
2		*Streptococcus bovies (ATCC-33317)*	8.6 ± 0.55	10.33 ± 0.58	12.25 ± 0.21	12.75 ± 0.17	18.2 ± 0.26
3	Gram - ve	*Salmonella typhimurium* (ATCC-14028)	8.33 ± 0.12	8.75 ± 0.96	10 ± 1.0	12.25 ± 0.19	16.83 ± 0.42
4		*Escherichia coli* (ATCC-35218)	7.72 ± 0.41	8.67 ± 0.58	9.83 ± 0.29	11.5 ± 0.26	17.4 ± 0.31

Values are means of triplicate determination ± standard deviation of mean

Table-3 Minimal inhibition concentration (MIC) (mg/mL) and minimal concentration (mg/mL) of essential oil and methanol extract of *A. absinthium L.*

Microorganisms	Essential oils		Methanol extract	
	MIC	MBC	MIC	MBC
Staphylococcus aureus (ATCC-29523)	0.097	> 0.39	0.78	> 1.56
Streptococcus bovies (ATCC-33317)	0.19	> 0.78	1.56	> 3.13
Escherichia coli (ATCC-35218)	0.39	> 1.56	3.1	> 6.25
Salmonella typhimurium (ATCC-14028)	0.39	> 0.78	1.56	> 3.13

Table-4 Anthelmintic activity of essential oil and methanolic extracts of the aerial part of *Artemisia absinthium*

Sample type	Concentration(mg/mL)	*Artemisia absinthium*	
		TTP in minutes	TTD in minutes
Control (DMSO)	-	-	-
Albendazole (standard)	25	2.9 ± 0.84	4.63 ± 0.8
Essential oil	25	4.78 ± 0.93	9.53 ± 0.16
	50	3.04 ± 0.1	6.88 ± 0.173
	75	2.77 ± 0.98	5.45 ± 0.76
	100	2.34 ± 0.52	4.49 ± 0.167
Methanoic extract	25	3.28 ± 0.26	6.89 ± 0.17
	50	3.07 ± 0.61	5.62 ± 0.37
	75	2.61 ± 0.41	4.92 ± 0.47
	100	2.04 ± 0.09	4.77 ± 0.73

Values of anthelmintic activity are represented as mean ± S.E.M., TTP = Time taken for Paralysis, TTD = Time taken for Death.

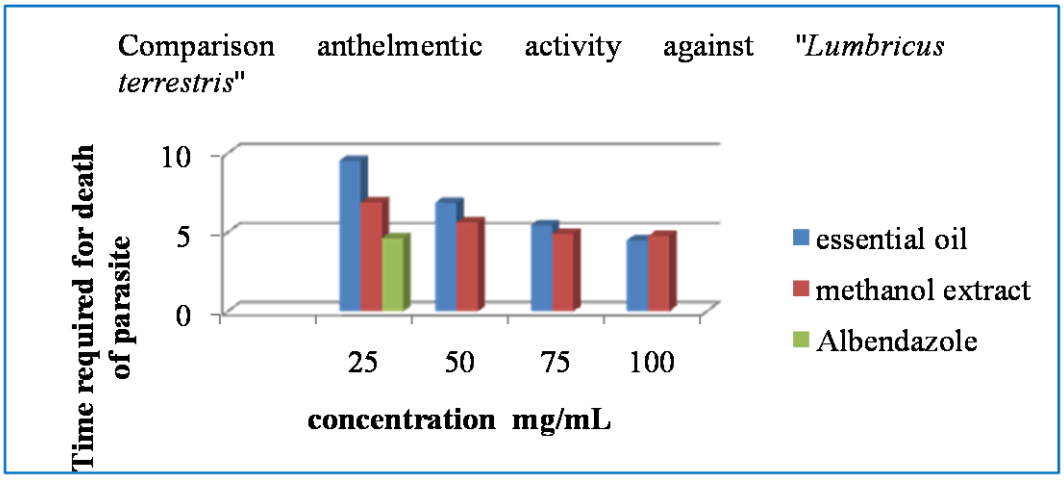

Figure-3. Comparison anthelmintic activities of essential oil and methanolic extract against *Lumbricus terrestris*

This observation confirmed the evidence given in previous study reporting that essential oil from medicinal plants contain more antibacterial substances than other extracts such as water, methanol, ethanol and hexane extracts[11]. The present results also indicated that the essential oil and methanoic extract of *Artemisia absinthium* showed almost same affective in against Gram-negative and Gram-positive bacteria.

Anthelmintic activities of the oils of *Artemisia absinthium*

The anthelmintic activity of the essential oil and methanoic extract of aerial part of *Artemisia absinthium* are presented in Table-4 and Figure-3.

Results were recorded as shown in Table-4 as in the form of time required for paralysis and death of parasite. At lower concentration longest time of paralysis and death of parasite was observed which may be because of low potency of essential oil and methanolic extract; but further increase in concentration were found to possess good anthelmintic potential in dose dependent manner as shown in observation Table-4.

Results were also plotted as shown in graph for comparison of potency of both essential oil and methanol extract. Both essential oil and methanol extracts were showing good anthelmintic activity when compared with

albendazole as standard, particularly at dose of 100 % concentration. But concentration of essential oil and methanol extract required to show equal potency with standard was very high. The results of study shows both the essential oil and methanoic extract showed anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with increasing concentrations. However, methanolic extract were slightly more effective as compared to essential oil. The possible explanation for better anthelmintic activity of methanolic extract compared to essential oil on larvae and adults parasites could be due to presence several anthelmintic bearing compounds like tannins, alkaloids, and flavonoids in methanolic extract than in the essential oil.

Acknowledgement

I would like to express my great gratitude to my brother Mr. Ephrem sahle for his support, prayers and financial assistance.

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